

DIGESTIVE PROCESSES OF HAEMATOPHAGOUS INSECTS

I. A LITERATURE REVIEW

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About 240 papers published between 1903 and early 1971, providing information on more than 150 species of haematophagous insects, are reviewed. Aspects of digestive physiology covered are size of the blood meals and their distribution within the alimentary canal, properties of the salivary glands, gross and histological changes in the gut and its contents, the enzyme content of the gut and the properties of the digestive enzymes. The relationship of digestive processes to vectoring ability is discussed briefly.

Environ 240 articles publiés entre 1903 et 1971 donnant des informations sur plus de 150 espèces d'insectes hématophages, sont révisés dans cet ouvrage. Les aspects de la physiologie de la digestion qui sont couverts sont: la quantité de sang que contiennent les repas et sa distribution dans le tractus alimentaire, les caractéristiques des glandes salivaires, les changements histologiques et l'ensemble des changements de l'intestin et de son contenu, le contenu enzymatique de l'intestin et les caractéristiques propres des enzymes digestives. La relation qui existe entre le processus digestif et l'aptitude qu'ont certains insectes à être vecteur de maladies est décrite brièvement.

The primary objectives of this paper are to summarize the knowledge of the digestive physiology of blood-sucking insects and to indicate some unsolved problems. Aspects considered include the size of the blood meals, the fate of these within the digestive tract, and the sources and nature of the enzymes involved in their breakdown. Sources of the blood meals and the mechanisms of ingestion and absorption are not considered except where these appear to have a bearing on digestion.

A comparative study of digestion in blood-sucking insects is of interest primarily for two reasons. First, blood feeding has evolved several times in the insects and a comparison of digestive processes may reveal that different species have overcome problems presented by the blood meal in different ways. Conversely, the nature of their food may have resulted in convergence in the digestive processes of different haematophagous species. A second and more practical reason is that many blood-sucking arthropods are vectors of pathogenic organisms. Since most of these pathogens spend some time in the gut of the vector, digestive processes of the insect could influence vectoring ability. The possible importance of the digestive processes of insects in studies of host relationships, nuisance created by blood-sucking insects, or the efficiency of disease transmission, has been suggested by several authors (West and Eligh, 1952; O'Gower, 1956; Detinova, 1962).

There are several general reviews of digestive physiology of insects (Day and Waterhouse, 1953a, b, c; Gilmour, 1961; House, 1965; Uvarov, 1929; Waterhouse, 1957; Waterhouse and Day, 1953; Wigglesworth, 1965). Related material has also appeared in articles by Barrington (1962), Fallis (1964), House (1958, 1961, 1962), Lipke and Fraenkel (1956), Snodgrass (1935), Vonk (1964), Waldbauer (1968) and Wigglesworth (1952). The digestive physiology of mosquitoes was reviewed briefly by Clements (1963) and this and the chapter on digestion in Wigglesworth (1965) constitute the most extensive reviews published on digestion by haematophagous insects. Chapters 20 and 22 in Christophers (1960) contain some relevant material but the discussion of digestion by adult mosquitoes (pp. 707-708) is very brief. Much of the work on digestion by tsetse flies was reviewed by Buxton (1955).

Within each of the following sections an attempt has been made to arrange the material more or less taxonomically; the exopterygotes (Hemiptera and Siphunculata) are discussed first followed by the Diptera (Nematocera, Brachycera, then Muscoid flies) and finally the Aphaniptera. However, to facilitate comparisons it occasionally has been necessary to deviate from this arrangement.

SIZE OF THE BLOOD MEAL

Several methods have been used to estimate blood meal size. The simplest and most widely employed involves weighing individuals before and immediately after feeding. Some workers have weighed batches of insects before and after feeding to obtain the average weight of the meal, particularly in studies involving very small insects, or in those undertaken with inadequate equipment. A further modification has been to compare the weights of batches of fed and unfed insects; this has been particularly useful when dealing with insects which will not readily feed under laboratory conditions, the data being obtained from field-caught insects. Blood meal volumes have usually been estimated by dividing the weight of the meal by the specific gravity of the blood. However in at least one case the volume was estimated by mixing midguts of blood-fed insects with a known volume of fluid and measuring the resulting volume (O'Connor and Beatty, 1937). This method gave an unusually low estimate of meal size. One difficulty encountered with all these procedures is that some species defecate during or immediately after feeding.

The first material defecated is generally from a previous meal or the serum of the meal just consumed (Boorman, 1960). Since erythrocytes are rarely defecated, tagging these with radioactive cesium or iron and then comparing radioactivity in the fed insect with aliquots of the host's blood has yielded estimates of the meal size which are usually not influenced by defecation during the act of feeding. Using the cesium tagging method the blood meal size of *Aedes aegypti* (L.) was estimated to be 4.21 μl compared with 2.47 to 2.71 μl by the gravimetric method (Boorman, 1960). A similar discrepancy in meal size was obtained with *Culex pipiens quinquefasciatus* Say (10 μl by Fe^{59} method, and 3.3 μl by gravimetric method) but not with *Triatoma infestans* (Klug) or *Panstrongylus megistus* (Burmeister) (de Freitas and Guedes, 1961). Chemical determination of the amount of hemoglobin (by conversion to alkaline hematin) in the engorged insect compared with the hemoglobin content of the host's blood (Kershaw et al, 1956) also yields estimates which are not influenced by defecation.

In some insects *Mansonia richiardii* (Ficalbi) and *Aedes cinereus* Meigen there is a correlation between pre-feeding weights and the amount of blood ingested, but in others (*Aedes cantans* (Meigen), *Aedes detritus* (Haliday) and *Aedes punctor* (Kirby)), there is not (Service 1968a). This may explain some of the variation in meal sizes reported by different workers for the same insect species. Environmental temperature apparently influences the amount of blood consumed by fifth instar *Triatoma dimiata* (Latreille) (174.5 mg at 26.5 C, 281.6 mg at 23 C) but not by other instars (Zeledon et al, 1970). The source of the blood meal may influence the quantity of blood ingested by *Pediculus humanus* L. (Krynski et al, 1952), *Cimex lectularius* L. (Johnson, 1937) and *A. aegypti* (Bennett, 1965). The physiological state of *Stomoxys calcitrans* (L.) may influence the size of the meal ingested (Anderson and Tempelis, 1970). *Glossina austeni* Newstead irradiated with 10 krad as pupae or 15 krad as teneral adults consumed as much blood as non-irradiated flies (Langley and Abasa, 1970). Environmental humidity does not influence the meal size of *Glossina tachinoides* Westwood (Buxton and Lewis, 1934) or *Glossina palpalis* (Robineau-Desvoidy) (Mellanby, 1936). In the latter species the second meal is larger than the first and older females tend to take

larger meals than young females (Mellanby, 1936).

The blood meal sizes for several species of insects are presented in Table I. The values recorded are the averages reported in the literature; where a range is given this represents the range of average meal sizes reported in the literature. Nymphal instars are indicated by Roman numerals.

The quantity of blood ingested by the insect is influenced by two physiological factors: a chemical in the blood which stimulates the insect to continue feeding and stretch receptors in the abdomen which inhibit further feeding. Adenosine-5'-phosphate and related compounds stimulate *Culex pipiens* (ssp) *pallens* Coquillett to engorge upon blood (Hosoi, 1959). Diphosphates and triphosphates of cytosine, guanine, inosine, and uridine; creatine phosphate, sodium pyrophosphate, riboflavin-5'-phosphate, 5'-adenylic acid and 3', 5'-cyclic adenylic acid stimulate *R. prolixus* to engorge (Friend, 1965; Friend and Smith, 1971). Termination of feeding by *R. prolixus* nymphs is apparently determined by stretch receptors in the abdomen (Maddrell, 1963). Nymphs whose nerve cord is severed between the pro- and mesothoracic ganglia will consume much larger meals than normal nymphs; nymphs with a fistula in the midgut (which permits draining the midgut during the act of feeding) will consume more blood over a longer period of time than the controls (Maddrell, 1963). By cutting the ventral cord of *A. aegypti* between various ganglia and then giving the mosquitoes a blood meal, Gwadz (1969) demonstrated that the quantity of blood ingested is determined by segmental stretch receptors. Similar, but less extensive, experiments led to the same conclusions for *A. taeniorhynchus*, *A. triseriatus*, *A. subalbus*, *C. pipiens quinquefasciatus* and *A. quadrimaculatus* (Gwadz, 1969). Lea (1967) reported that ablation of the median neurosecretory cells did not affect the amount of blood ingested by *A. taeniorhynchus*, *A. sollicitans* or *A. triseriatus* but reduced the blood meal size of *A. aegypti* by 35%.

The nature of the hunger mechanism has not been elucidated but it has been suggested that some blood-sucking insects have an optimal frequency of feeding (Gooding, 1960). Such an optimum could result from an interaction of meal size with the rates of digestion, absorption, and utilization of some component of the meal. The respiratory rate of *G. morsitans* reaches a maximum about 24 hours after feeding on a guinea-pig. At 12, 24, and 48 hours after feeding there is a high correlation between the meal size and the respiratory rate. Rajagopal and Bursell (1966) interpreted this increased oxygen consumption as being linked to the metabolic processes associated with digestion, absorption, deamination, detoxication, uric acid synthesis and excretion.

DISTRIBUTION OF MEALS WITHIN THE ALIMENTARY CANAL

In the lice, fleas, and blood-sucking bugs the digestive tract has no oesophageal diverticula and the blood meal is conveyed directly to the midgut. Blood is stored in the expanded anterior part of the midgut in bugs and is digested only in its posterior reaches (Bacot, 1915; Wigglesworth, 1936).

Flies, however, have from one to three oesophageal diverticula. Mosquitoes usually dispatch sugar solutions to the diverticula and blood to the midgut. The subject has been reviewed by Trembley (1952) and Megahed (1958). Trembley also presented data on the distribution of blood and sugar solutions in the various parts of the alimentary canal of 9 species of mosquitoes (*Anopheles freeborni* Aitken, *Anopheles aztecus* Hoffman, *Anopheles quadrimaculatus*, *Anopheles albimanus*, *Aedes albopictus* Skuse, *Aedes aegypti*, *Aedes atropalpus* (Coquillett) *Aedes vexans*, and *Culex pipiens* Linnaeus). The effects of interrupted feedings, time after feeding and the method of obtaining the blood meal (i.e. from a droplet

Table 1A. Blood meal sizes of some hemimetabolous insects.

Species	Stage					Sex		References
	I	II	III	IV	V	♂	♀	
<i>Pediculus humanus</i> L.	-	-	-	-	-	-	0.85-1.2	Buxton, 1947; Kryński <i>et al.</i> , 1952
<i>Cimex hemipterus</i> Fabricius	0.5	1.3	3.1	4.5	6.8	2.6	5.8	Wattal and Kalra, 1961
<i>Cimex lectularius</i> L.	0.26 -0.9	0.48 -1.7	1.09 -2.9	2.86 -5.0	5.53 -10.1	3.81 -6.2	6.48 -9.47	Jones, 1930; Johnson, 1937 Tawfik, 1968
<i>Panstrongylus megistus</i> (Burmeister)	4.*	-	-	-	-	-	-	de Freitas and Guedes, 1961
<i>Rhodnius prolixus</i> Stål	3.2 -5.9	13.8 -15.8	38.3 -48.7	108 -127	237 -284	130	187 -210	Buxton, 1930; Friend <i>et al.</i> , 1965 Goodchild, 1955; Pippin, 1970
<i>Triatoma dimiata</i> (Latreille)	4.5 -5.4	11.1 -13.3	42.3 -47.1	87.6 -89.5	174 -282	220	283	Zeledón <i>et al.</i> , 1970
<i>Triatoma gerstaeckeri</i> (Stål)	3.9 -4.0	7.4 -8.6	27.4 -34.4	56.8 -61.2	186 -192	133	218	Pippin, 1970; Thurman, 1945
<i>Triatoma infestans</i> (Klug)	1.5	4.0	10.0	107 -186	195 -382	440*	307	de Freitas and Guedes, 1961 Goodchild, 1955
<i>Triatoma sanguisuga</i> (LeConte)	-	-	-	-	-	43	61	Hays, 1965
<i>Triatoma sanguisuga texana</i> Usinger	1.2	4.2	11.6	46.2	129.7	52.6	79.4	Pippin, 1970

* Indicates a μ l value, all others are in mg.

Table 1B. Blood meal size of female mosquitoes.

Species	Meal size	References
<i>Aedes aegypti</i> (L.)	2.1- 4.5 mg	Garnham, 1947; Gwadz, 1969; Howard, 1962; Jeffery, 1956; Lea, 1967; Roy, 1936
	1.6- 4.2 μ l	Bennett, 1965; Boorman, 1960; Jeffery, 1956
<i>Aedes cantans</i> (Meigen)	5.9 mg	Service, 1968a
<i>Aedes cinereus</i> Meigen	2.6 mg	Service, 1968a
<i>Aedes detritus</i> (Haliday)	3.5 mg	Service, 1968a
<i>Aedes hexodontus</i> Dyar	\leq 5.5 mg	Barlow, 1955
<i>Aedes infirmatus</i> Dyar and Knab	4.9 mg	Woodard and Chapman, 1965
<i>Aedes punctator</i> (Kirby)	4.0 mg	Service, 1968a
<i>Aedes sollicitans</i> Walker	3.9- 8.5 mg	Lea, 1967; Woodard and Chap- man, 1965
<i>Aedes sticticus</i> (Meigen)	2.1 mg	Stage and Yates, 1936
<i>Aedes taeniorhynchus</i> (Wiedemann)	3.2- 3.8 mg	Gwadz, 1969; Lea, 1967; Woodard and Chapman, 1965
<i>Aedes triseriatus</i> (Say)	3.93 mg	Gwadz, 1969
<i>Aedes vexans</i> (Meigen)	2.1- 4.7 mg	Stage and Yates, 1936; Woodard and Chapman, 1965
<i>Anopheles albimanus</i> Wiedemann	2.6 mg	Jeffery, 1956
<i>Anopheles litoralis</i> King	2.1 mg	Laurel, 1934
<i>Anopheles ludlowae</i> (Theobald)	2.1 mg	Laurel, 1934
<i>Anopheles maculatus</i> Theobald	2 mg	Laurel, 1934
<i>Anopheles minimus</i> Theobald	1 mg	Laurel, 1934
<i>Anopheles quadrimaculatus</i> Say	2.3- 5.5 mg	Gwadz, 1969; Jeffery, 1956; Woodard and Chapman, 1965
<i>Armigeres subalbus</i> (Coquillett)	3.7 mg	Gwadz, 1969
<i>Culex pipiens quinquefasciatus</i> Say	2.0- 5.0 mg	Rachou <i>et al.</i> , 1957; Wharton, 1960; Jordon and Goatly, 1962; Gwadz, 1969
	1.0-10. μ l	O'Connor and Beatty, 1937; de Freitas and Guedes, 1961
<i>Culex salinarius</i> Coquillett	2.1 mg	Woodard and Chapman, 1965
<i>Culiseta annulata</i> (Schrank)	7.0 mg	Service, 1968a
<i>Culiseta inornata</i> (Williston)	4.5 μ l	Owen and Reinholz, 1968
<i>Mansonia perturbans</i> (Walker)	4.3 mg	Woodard and Chapman, 1965
<i>Mansonia richiardii</i> (Ficalbi)	3.3 mg	Service, 1968a
<i>Psorophora ciliata</i> (Fabricius)	25.0 mg	Woodard and Chapman, 1965
<i>Psorophora confinnis</i> (Lynch Arribalzaga)	6.0 mg	Woodard and Chapman, 1965
<i>Psorophora cyanescens</i> (Coquillett)	9.2 mg	Woodard and Chapman, 1965
<i>Psorophora ferox</i> (Humboldt)	5.1 mg	Woodard and Chapman, 1965

Table 1C. Blood meal sizes of some flies. Data are from females except where otherwise indicated.

Species	Meal size	References
Ceratopogonidae		
<i>Phlebotomus papatasi</i> Scopoli	0.1 mg	Adler and Theodor, 1926
<i>Leptoconops kerteszi</i> Kieffer	0.23 mg	Foulk, 1967
Simuliidae		
<i>Prosimulium decemarticulatum</i> (Twinn)	2.2 μ l	Bennett, 1963
<i>Simulium aureum</i> Fries	2.9 μ l	Bennett, 1963
<i>Simulium croxtoni</i> Nicholson and Mickel	3.26 μ l	Bennett, 1963
<i>Simulium damnosum</i> Theobald	1.08 μ l	Crosskey, 1962
<i>Simulium latipes</i> (Meigen)	2.65 μ l	Bennett, 1963
<i>Simulium quebecense</i> Twinn	2.14 μ l	Bennett, 1965
<i>Simulium rugglesi</i> Nicholson and Mickel	1.36 mg 1.94 μ l	Anderson <i>et al.</i> , 1962; Bennett, 1963
Tabanidae		
<i>Chrysops dimidiata</i> Van der Wulp	19.7 μ l	Kershaw <i>et al.</i> , 1956
<i>Chrysops silacea</i> Austen	24.2 - 30 μ l	Gordon and Crewe, 1953; Kershaw <i>et al.</i> , 1956
<i>Hybomitra frontalis</i> (Walker) (= <i>T. septentrionalis</i>)	40 mg 27 mg	Kershaw <i>et al.</i> , 1954 Miller, 1951
<i>Hybomitra affinis</i> (Kirby)	156 mg	Miller, 1951
<i>Tabanus quinquevittatus</i> Wiedemann	71 mg	Tashiro and Schwardt, 1949
<i>Tabanus sulcifrons</i> Macquart	344 mg	Tashiro and Schwardt, 1949
Muscidae		
<i>Glossina austeni</i> Newstead (δ)	11.4 mg	Langley and Abasa, 1970
<i>Glossina austeni</i> Newstead (f)	19.9 mg	Langley and Abasa, 1970
<i>Glossina brevipalpis</i> Newstead (δ)	107. mg	Moloo and Kutuza, 1970
<i>Glossina morsitans</i> Westwood (δ)	34 mg	Lester and Lloyd, 1928
<i>Glossina morsitans</i> Westwood (f)	37 mg	Lester and Lloyd, 1928
<i>Glossina palpalis</i> (Robineau-Desvoidy) (δ)	22.74- 33.70 mg	Mellanby, 1936
<i>Glossina palpalis</i> (Robineau-Desvoidy) (f)	25.13- 41.67 mg	Mellanby, 1936
<i>Glossina tachinoides</i> Westwood (δ)	30 mg	Lester and Lloyd, 1928
<i>Glossina tachinoides</i> Westwood (f)	28 mg	Lester and Lloyd, 1928
<i>Glossina tachinoides</i> Westwood (?)	8.94- 14.00 mg	Buxton and Lewis, 1934
<i>Stomoxys calcitrans</i> (L.) (δ)	6.9 - 9.45 mg	Anderson and Tempelis, 1970; Suenaga, 1965
<i>Stomoxys calcitrans</i> (L.) (f)	10.5 - 16.43 mg	Anderson and Tempelis, 1970; Suenaga, 1965
<i>Stomoxys calcitrans</i> (L.) (?)	25.8 mg	Parr, 1962

or through a membrane) were investigated. The results showed that the major factor in determining the destination of the meal in the alimentary canal was its composition. Similar results were reported for *Phlebotomus papatasi* (Adler and Theodor, 1926), *Simulium damnosum* (Lewis, 1953), *Simulium venustum* Say (Yang and Davies, 1968b), *Culicoides nubeculosus* Meigen (Megahed, 1956, 1958), *Chrysops* and *Tabanus* (Wigglesworth, 1931). Although *Anopheles maculipennis* Meigen generally dispatched blood to the midgut only, about 1/3 of the mosquitoes also had some blood in the ventral diverticulum (Wright, 1924). It has been claimed that *A. aegypti* but not *A. albimanus* (reported as *Anopheles tarsimaculatus*) passed blood first into the diverticula and then within half an hour of feeding into the midgut (Pawan, 1937). A honey - citrated blood mixture fed to *A. maculipennis*, *C. pipiens*, and *A. aegypti* (recorded as *Aedes argenteus*) went to the diverticula. The ventral diverticulum filled first, followed by the dorsal diverticula after unusually large meals (MacGregor and Lee, 1929). Radiographic studies *in situ* of the distribution of sugar solutions and blood meals in the digestive tract of *A. aegypti* showed that sugar meals could be moved from one diverticulum to another and that during ingestion of a blood meal the hind most part of the midgut filled first (Guptavanij and Venard, 1965).

Among some flies however, (*Glossina* sp., Wigglesworth, 1931; *Stomoxys calcitrans*, Champlain and Fisk, 1956; *Hippelates pallipes* (Loew), Kumm, 1932) the blood meal goes first to the oesophageal diverticulum and then to the midgut. In the eye gnat, *H. pallipes*, only a small quantity of blood goes directly to the midgut. Within 30 mins of feeding the gnats begin transferring blood from the crop to the midgut and by 6 hours after feeding about half of the meal is transferred. The blood blackens in the midgut as digestion takes place. By 2 days after feeding the crop is empty (Kumm, 1932). The first portion of a blood meal ingested by tsetse flies (*Glossina morsitans* and *Glossina tachinoides*) is conveyed to the midgut where it is localized in 3 regions, a clot forming in the most posterior. Additional blood is dispatched to the crop (oesophageal diverticulum) but it does not clot there. By 2.5 hours after feeding most of the blood passes from the crop into the midgut which is now more or less continuously full of blood. The blood at the posterior end of the midgut clots and progressively darkens while the blood in the anterior of the midgut does not clot and is bright red. By 24 hours after the meal the crop is empty and the blood in the midgut is again divided into regions by folds in the gut. The blood in the anterior part of the midgut forms a pasty mass as a result of absorption of most of the serum but a true clot does not form. The blood cells in this region of the midgut are contained in a mucilaginous secretion from the gut cells. By 48 hours the blood mass in the gut greatly decreases and the dark mass progressively contracts posteriorly (Lester and Lloyd, 1928).

When blood only is fed to *A. aegypti* it is dispatched to the midgut in all individuals, traces being found in the diverticula of only 6% of the mosquitoes (Day, 1954). Increasing the concentration of glucose in a blood-glucose mixture increases the frequency with which meals go to the diverticula. At concentrations above 0.46 M glucose all mosquitoes send the meal to the diverticula, only 7 to 10% also convey food to the midgut. These results indicate that there are receptors in this mosquito for both glucose and some component of blood. *A. aegypti* can detect sucrose and probably arabinose, mannose, and raffinose but not lactose when mixed with blood. Although both plasma and erythrocytes are detected solutions of haemoglobin and albumin with glucose go mainly to the diverticulum. Mosquitoes feeding on very dilute erythrocyte suspensions in sugar dispatch the sugar solution to the diverticulum and the erythrocytes to the midgut. This is apparently accomplished by a group of spines in the neck of the diverticulum which are capable of acting as a sieving mechanism when particles are sparse. Experiments with red cell ghosts and fly sarcosomes in water indicate that the particulate nature of the erythrocytes is one factor in blood detected by

the mosquito. Day (1954) explained the distribution of fluids by proposing that the pit organs in the buccal cavity detect sugars and the resulting impulses mediate the relaxation of the diverticula sphincter muscles. Similarly, the papillar sense organs detect components of the blood and relaxation of the cardiac sphincter permits blood to enter the midgut.

Hosoi (1954) reported upon the mechanism by which *Culex pipiens* (issp) *pallens* distributes fluids to the midgut and diverticula. As in other mosquitoes, sugary solutions go to the diverticula and blood to the midgut. Dilute suspensions of erythrocytes in saline go to the midgut but if glucose is added to the meal there is an increased tendency to dispatch the meal to the diverticula. Replacing the erythrocytes with other particulate matter in a 5% glucose solution generally results in the meal being dispatched to the diverticula - thus indicating that the particulate nature of a blood meal is not the only stimulus for dispatching food to the midgut. Hosoi suggested that erythrocytes have, adsorbed to their surfaces, substances which are responsible for stimulating certain sense organs. Sensory receptors on the labium respond to glucose but not to erythrocytes while the reverse is true for receptors on the fascicle. However the existence of other sense organs capable of differentiating the meal composition was not excluded.

Theories concerning the biological importance of the retention of sugars in the diverticula were reviewed by Trembley (1952), Megahed (1958), and Christophers (1960, p. 489). Two of these theories pertain to digestive physiology. One is that the carbohydrate meal is stored in the diverticula so that the hunger mechanism, which is presumed to originate in the midgut, is not interfered with, and the mosquito is thus always ready to take blood (Day, 1954). The other is that by storing carbohydrate solutions in impervious structures the mosquito carries with it a supply of water which may be passed to the midgut for absorption as needed. If the second theory is correct, then the mosquito's physiology is adapted to conserving water from a carbohydrate meal while disposing of much of the water in a blood meal (Boorman, 1960; Howard, 1962). Denisova (1949) investigated the function of horsefly diverticula by injecting water and salt solutions into the flies. She concluded that the diverticula store water which is supplied in small amounts to the midgut minimizing rapid drops in haemolymph osmotic pressure. However, it seems doubtful that nectar solutions, with their high sugar content, would significantly lower the osmotic pressure of insect haemolymph if dispatched directly to the midgut.

The inhibition of honeybee proteolytic enzymes by honey (Bailey, 1952) suggests that nectar may contain substances which inhibit insect proteinases. The diverticula of insects which consume both nectar and blood may thus function as a mechanism for separating inhibitors present in one type of meal from the digestive enzymes required to digest another type of meal. MacGregor (1930) stated that "Poisonous fluids invariably enter the diverticula" of mosquitoes and gave as an example the ingestion of 20% formalin. The mosquitoes died immediately after the diverticula filled and traces of formalin passed into the midgut. This was interpreted as indicating that absorption did not take place from the diverticula.

THE SALIVARY GLANDS AND THEIR SECRETIONS

Since saliva is usually the first insect secretion to which the blood meal is exposed there has been considerable interest in the effects of saliva upon blood. Bates (1949) suggested that anticoagulins and haemagglutinins from the salivary glands of mosquitoes assist in the preliminary breakdown of the blood meal. Fisk (1950) suggested that coagulation or agglutination of the blood meal denatures its proteins sufficiently to permit attack by mosquito proteinases. Regrettably these suggestions have not been investigated.

Aedes aegypti feeding upon suckling mice left an average of 4.7 μg of saliva in the mice

during consumption of a meal (Devine et al, 1965). Probably because of the minute amount of saliva secreted by blood-sucking insects, most investigators have used homogenates of the salivary glands to test for a variety of enzymes, haemolysins, agglutinins, and anticoagulins. The most commonly encountered are agglutinins and anticoagulins; a summary of their occurrence among the blood-sucking insects is given in table 2.

The ability of salivary gland emulsions to cause agglutination sometimes depends upon the source of the erythrocytes. *A. maculipennis* salivary glands agglutinate erythrocytes from man, donkey, rabbit, and dogs, but not those from mice, guinea-pigs, or monkey (York and Macfie, 1924; confirmed for white mice by Shute, 1935). The agglutinin from *A. quadrimaculatus* is effective against red blood cells of man, mule, cow, pig, dog, rabbit, guinea-pig, rat, and mouse but not chicken or turtle (Metcalf, 1945).

Agglutinins are restricted to the median acinus of the salivary glands of *A. maculipennis* (de Buck 1937) and *A. quadrimaculatus* (Metcalf, 1945) but occur in all three acini of *C. annulata* salivary glands (de Buck 1937). Solutions of these agglutinins are heat labile (York and Macfie, 1924; de Buck 1937; Metcalf, 1945). The agglutinin from *A. maculipennis* was reported by York and Macfie (1924) to be inactivated by desiccation, but by de Buck (1937) to be stable for several months at room temperature or for 1 hour at 99 C when dried. Similar properties for the agglutinin from *C. annulata* were reported by de Buck (1937).

Baptist (1941) reported that the anticoagulin in *R. prolixus* salivary glands was inactivated by heating to 70 C but Hellmann and Hawkins (1964) reported this anticoagulin was stable at 60 or 80 C for 30 mins, was not affected by 0.1 N HCl or 0.1 N NaOH at R.T. or 60 C for 30 mins but was completely destroyed by heating to 100 C for 5 mins. It was not precipitated by centrifuging at 100,000 g for 30 mins but was removed from solution by dialysis in the cold. This anticoagulin (designated Prolixin-S by Hellmann and Hawkins, 1965) did not inhibit thrombin, but acted mainly upon factor VIII (the antihæmophilic factor). Prolixin-S retained its activity upon freezing, freeze drying or dialysis but was inactivated by trypsin.

Anticoagulin cannot be detected in *R. prolixus* midgut immediately after feeding but is found 4 hours later. Fractionation of the gut contents indicate that the anticoagulin is probably present but inhibited by some component of the blood meal (Hellmann and Hawkins, 1965). A salivary gland anticoagulin from *T. rubofasciata* is inactivated by normal rabbit serum but not by serum from a rabbit used as a host for one year (Cornwall and Patton, 1914). A salivary gland anticoagulin from *T. maculata* is not an anti-thrombin and is slightly less heat stable than that of *R. prolixus* (Hellmann and Hawkins, 1966).

Anticoagulin occurs in all three acini of the salivary glands of *C. pipiens* (de Buck 1937), *A. quadrimaculatus* (Metcalf, 1945), *A. plumbeus* and *A. maculipennis* (de Buck, 1937). However, in the latter species its concentration in the lateral acini is very low. The anticoagulin from *A. maculipennis* is stable at R.T. for several months when dried and is heat stable as either a desiccated preparation (99 C for 1 hr) or saline solution (100 C for 35 mins) (de Buck, 1937). Anticoagulins from *C. pipiens* and *C. annulata* are stable when desiccated but are more heat labile than the anticoagulin from *A. maculipennis* (de Buck 1937). The *A. quadrimaculatus* anticoagulin is thermostable (Metcalf, 1945).

The anticoagulins from the salivary glands of *G. tachinoides* and *G. morsitans* are non-dialyzable and that of the former species is not markedly affected by 0.1 N KOH or 0.1 N HCl. They are stable at temperatures up to 90 C but at this temperature lose half their activity in 15 mins and all activity in 30 mins; at 100 C all activity is lost in 15 mins. From experiments with fibrinogen solutions and citrated blood, Lester and Lloyd (1928) suggested that the tsetse fly anticoagulin is an antikinase. They found that although the delay

Table 2. Agglutinins, coagulins and anticoagulins in some blood-sucking insects.

Species	Salivary Glands		Midgut		References
	Agglutinin	Anticoagulin	Coagulin	Anticoagulin	
Lice					
<i>Pediculus humanus</i> L.		+			Nuttall, 1917a
<i>Phthirus pubis</i> (L.)		+ ¹	+ ²		Grütz, 1923
Bugs					
<i>Cimex lectularius</i> L.		+			Puri, 1924; Baptist, 1941
<i>Cimex hemipterus</i> Fabricius (= <i>Cimex rotundatus</i>)		-			Cornwall and Patton, 1914
<i>Rhodnius prolixus</i> Stål		+			Baptist, 1941
<i>Triatoma infestans</i> (Klug)		+		+	Hellmann and Hawkins, 1964, 1965
<i>Triatoma maculata</i> (Erichson)		+		+	Baptist, 1941
<i>Triatoma rubrofasciata</i> (DeGeer)		+		+	Hellmann and Hawkins, 1966
					Cornwall and Patton, 1914
Sandflies					
<i>Phlebotomus papatasi</i> Scopoli	-	+		+	Adler and Theodor, 1926
Mosquitoes					
<i>Aedes aegypti</i> (L.)	-	-			Yorke and Macfie, 1924; Metcalf, 1945
	-	-			McKinley, 1929
<i>Aedes aegypti</i> (= <i>Aedes calopus</i>)	-	-			Gorden and Lumsden, 1939
<i>Aedes detritus</i> (Haliday)	-	-			Shute, 1935
<i>Aedes rusticus</i> (Rossi)	-	-			Shute, 1935
<i>Aedes vexans</i> (Meigen)	-	-			Shute, 1935
					Metcalf, 1945

Present +, not found -, those not checked for are left blank; ¹ present in head and thorax, ² present in abdomen.

Table 2 (continued)

Species	Salivary Glands		Midgut		References
	Agglutinin	Anticoagulin	Coagulin	Anticoagulin	
Mosquitoes (continued)					
<i>Anopheles claviger</i> (Meigen)	-				Shute, 1935
<i>Anopheles crucians</i> Wiedemann	+	+			Metcalf, 1945
<i>Anopheles jamesii</i> (Theobald)	+	+			Cornwall and Patton, 1914
<i>Anopheles labranchiae atroparvus</i> VanThiel (= <i>An. m. var. atroparvus</i>)	+				Shute, 1935
<i>Anopheles maculatus</i> (Theobald)	-				Shute, 1935
<i>Anopheles maculipennis</i> Meigen	+	+	-	+	Yorke and Macfie, 1924 de Buck <i>et al.</i> , 1932 de Buck, 1937 Shute, 1948
<i>Anopheles maculipennis</i> Meigen var. <i>maculipennis</i>	+				Shute, 1935
<i>Anopheles maculipennis</i> var. <i>messeae</i> Falleroni	+				Shute, 1935
<i>Anopheles plumbeus</i> Stephens	-				Shute, 1935
<i>Anopheles punctipennis</i> (Say)	-	+			de Buck, 1937
<i>Anopheles quadrimaculatus</i> Say	+	+			Metcalf, 1945
<i>Anopheles rossi</i> Giles	+	+			Metcalf, 1945
<i>Anopheles stephensi</i> Liston	-				Cornwall and Patton, 1914
<i>Anopheles subpictus</i> Grassi	+	+			Shute, 1948
<i>Culex pipiens quinquefasciatus</i> Say	-				Cornwall and Patton, 1914
<i>Culex pipiens</i> L.	-				Shute, 1935
	-	+	+		Nuttall and Shipley, 1903 Yorke and Macfie, 1924 Shute, 1935 de Buck, 1937

Table 2 (continued)

Species	Salivary Glands		Midgut		References
	Agglutinin	Anticoagulin	Coagulin	Anticoagulin	
Mosquitoes (continued)					
<i>Culex restuans</i> Theobald	-	-			Metcalf, 1945
<i>Culex salinarius</i> Coquillett	-	-	+		Metcalf, 1945
<i>Culiseta annulata</i> (Schrank)	+	+			de Buck <i>et al.</i> , 1932; de Buck, 1937
	-	-			de Buck, 1937
	-	-			Shute, 1935
	-	-			Yorke and Macfie, 1924
<i>Psorophora discolor</i> (Coquillett)	-	-			Metcalf, 1945
<i>Mansonia richiardii</i> (Ficalbi)	-	-			Shute, 1935
Horse flies					
<i>Tabanus albimediis</i> Walker	+				Cornwall and Patton, 1914
Muscoid flies					
<i>Gasterophilus intestinalis</i> (DeGeer)		+			Tatchell, 1958
<i>Glossina morsitans</i> Westwood	+	+	+	+	Lester and Lloyd, 1928
<i>Glossina tachinoides</i> Westwood	+	+			Yorke and Macfie, 1924
	+	+			Lloyd, 1928
	+	+	+	+	Lester and Lloyd, 1928
<i>Hippobosca</i> L. sp.	-	-			Wigglesworth, 1930
<i>Musca convexifrons</i> Thomson	-	-	+		Cornwall and Patton, 1914
<i>Musca crassirostris</i> Stein			+		Cornwall and Patton, 1914
(= <i>Philoematomyia insignis</i>)	+	+	+	-	Cornwall and Patton, 1914
<i>Musca nebulosa</i> Wiedemann	-	-	-		Cornwall and Patton, 1914
<i>Musca pattoni</i> Austen	+	+	+		Cornwall and Patton, 1914
<i>Stomoxys calcitrans</i> (L.)	-	-	+		Cornwall and Patton, 1914
<i>Stomoxys</i> (probably <i>indica</i> Picard)	-	-	+		Cornwall and Patton, 1914

in clotting of sheep's blood is a function of the number of glands present, a simple straight line relationship is not obtained.

The anticoagulin in the salivary glands of *M. crassirostris* (reported as *Philoematomyia insignis*) is only slightly inactivated by heating to 100 C for 10 mins. This anticoagulin is probably non-antigenic in rabbits and rats. However normal sera of calf, rat, and rabbit contain one or more substances which inactivate the anticoagulin. Unlike sodium citrate inhibition of coagulation, the anticoagulin from *M. crassirostris* is not overcome by addition of CaCl_2 . Anticoagulin activity of female salivary glands is greater than that of males. Newly emerged adults appear to have less anticoagulin than older adults which have had an opportunity to feed (Cornwall and Patton, 1914).

Hemolytic activity of salivary gland emulsions has never been demonstrated, although it has been tested for in *C. pipiens* (Nuttall and Shipley, 1903; Yorke and Macfie, 1924), *A. aegypti* (Yorke and Macfie, 1924; McKinley, 1929), *C. annulata*, and *G. tachinoides* (Yorke and Macfie, 1924), and *P. papatasi* (Adler and Theodor, 1926).

Although salivary glands have frequently been examined for digestive enzymes these have rarely been demonstrated. Salivary glands of *C. lectularius*, *R. prolixus*, and *T. infestans* do not have demonstrable amounts of protease, lipase, invertase, or amylase (Baptist, 1941). Although esterases occur in the salivary gland tissues of *Anopheles freeborni* and *A. aegypti* they are not demonstrable in the salivary secretions (Frevogel, Hunter and Smith, 1968). Roy (1937) examined the salivary glands of *G. intestinalis* and found no amylase, lipase, maltase, lactase, pepsin or trypsin, but he did find a milk-clotting (proteolytic) enzyme. The existence of this milk clotting enzyme was confirmed by Tatchell (1958) who also demonstrated invertase, maltase, and amylase; the later with a pH optimum at 6. Wigglesworth (1929) reported that *Glossina morsitans submorsitans* Newstead and *G. tachinoides* salivary glands did not contain amylase, invertase, maltase, lactase, trypsin, pepsin, or peptidase. However, the salivary glands of *Glossina austeni* contain a factor which activates plasminogen (Hawkins, 1966). This or a similar material is also found in the crop, midgut, and hindgut where presumably it contributes to the lysis of the blood clot. A weak amylase occurs in *S. calcitrans* salivary glands (Champlain and Fisk, 1956). However, Roston and Gamal-Eddin (1961) found no evidence for amylase or proteinase (active at pH 6, 7, or 8.3) in the salivary glands of *S. calcitrans*, *Stomoxys sitchensis* Rondani, or *Musca vitripennis* Meigen. The salivary glands of larvae of *Protocalliphora avium* Shannon and Dobrosky (reported as *Apaulina avium*) contain butyrase and a weak maltase (Rockstein and Kamal, 1954).

Metcalf (1945) found no evidence of protease, lipase, amylase, or lecithinase-A in the salivary glands of *A. quadrimaculatus*. Wigglesworth (1931) reported that *Chrysops* and *Glossina* salivary glands contained a lipase. No invertase occurs in the salivary glands of *Prosimulium fuscum* Syme & Davies, or *Simulium venustum* (Yang and Davies, 1968c).

No cytological changes were detected in the salivary glands of *A. aegypti* immediately after feeding on sugar or human blood but histochemical changes observed in the glands during the 24 hours following a blood meal led to the conclusion that "feeding depletes the glands and that this depletion leads to the resynthesis of secretory products" (Orr, Hudson and West, 1961).

The salivary glands of female blackflies (probably *S. venustum*) contain a Periodic acid-Schiff positive material prior to the time a blood meal is taken. This material is present in only small amounts in the glands immediately after feeding, but reappears during the next 96 hours (Gosbee, Allen, and West, 1969).

A major function of anticoagulins in saliva appears to be the prevention of premature clotting of the blood meal. Lloyd (1928) reported that removal of the salivary glands of *G.*

tachinoides did not prevent feeding on humans, but sooner or later the flies died with a blood clot in the proboscis or oesophagus. *Aedes stimulans* (Walker) whose salivary ducts had been cut ingested blood in a normal manner and developed eggs. No observable difference between the blood clot in the midgut of operated and control mosquitoes could be detected (Hudson, Bowman, and Orr, 1960).

GROSS CHANGES IN QUANTITY & QUALITY OF THE GUT CONTENTS.

Mosquitoes feeding on chicks with heavy *Plasmodium gallinaceum* Brumpt infections may defecate 20 to 50% of the meal within a few hours (Howard, 1962). The discharge of blood during and following feeding seems to favour the elimination of gametocytes thus reducing the intensity of the infection in the mosquitoes (Mitzmain, 1917). When undisturbed *Anopheles gambiae* Giles and *Anopheles funestus* Giles will engorge until blood is passed from the anus; sometimes the amount of blood passed is at least half the volume ingested (Hocking and MacInnes, 1949). Neither the coagulation time nor the blood corpuscles are affected by this rapid passage through these mosquitoes. The fluid defecated by *Aedes aegypti* during and just after feeding contains uric acid, simple proteins or amino acids, and occasionally red blood corpuscles but no reducing sugars (Boorman, 1960). The volume of the fluid passed is approximately $1.5 \mu\text{l}$; some of this comes from the serum while the remainder (actually the first few drops passed) containing uric acid, is probably present in the hind gut at the time of feeding.

The discharge of material from the anus during and just after feeding has been observed in *Pediculus humanus* (Nuttall, 1917b), tsetse flies (Lester and Lloyd, 1928), and *Culicoides nubeculosus* (Megahed, 1958).

The relatively great changes in the size and shape of the abdomens of some blood-sucking insects occurring during feeding and the translucence of the abdominal pleura have permitted observations on digestion with no more than a dissecting scope or hand lens. Frequently, these observations could be made in more detail by simply dissecting the insect and without the use of elaborate histological techniques.

Sella (1920a) divided the digestion of blood and the development of the ovaries into 7 stages. These stages were summarized by Detinova (1962, p. 57) who also reviewed aspects of blood digestion relating to age-grouping methods and studies of ovarian development. Modification of Sella's (1920a) method have been developed by several workers (Hocking and MacInnes, 1949 for *Anopheles*; Jackson, 1933 for *Glossina*; Linley, 1965 for *Leptocnops*).

Blood ingested by *Culex pipiens quinquefasciatus* (*C. fatigans*) turns black within 6 hours and the abdomen is $3/4$, $2/3$, $1/2$, slightly less than $1/2$, and less than $1/3$ distended at 12, 24, 36, 48 and 60 hours after a blood meal (O'Gower, 1956). In the tropics *Anopheles* species generally take 2 days to digest a blood meal and develop eggs but *Anopheles vagus* Dönnitz requires only 24 hours to accomplish these same processes in Assam, India (Muirhead-Thomson, 1951).

Chicken blood ingested by *A. aegypti* clots in about 30 minutes compared to 25 minutes for the same volume on a glass slide (Howard, 1962). By 45 minutes a clear yellowish border forms around ingested blood and this adheres to the surfaces of both the midgut cells and the blood meal. This adherence persists for about 12 hours by which time the peritrophic membrane is being formed. By 24 hours the meal becomes a comparatively firm clot, and although its volume decreases its consistency does not change after that time. By 72 hours after feeding the midgut is usually empty.

Gross changes in the appearance of the blood meal of *A. aegypti* fed on a rat have

been reported by Akov (1965). The blood is bright red immediately after ingestion. As digestion proceeds inwards from the periphery the blood meal turns brown. By 24 hours the meal is brown except for the center. Akov considered digestion to be complete and elimination of the residue to begin when the color of the blood was brown throughout. On this basis digestion is complete and defecation begins by 36 hours. The size of the blood clot decreases and the midgut wall becomes folded. By 42 hours after feeding 2/3 of the females have empty midguts and 1/3 contain small residues of blood. By 48 hours about 1/4 of the mosquitoes contain residues in the midgut or hindgut. Elimination of blood residues is complete in all mosquitoes by 54 hours after feeding.

Feeding increasing concentrations of 5-fluorouracil (5-FU) to *A. aegypti* for 2 days prior to a blood meal results in an increasing tendency for the mosquitoes to retain undigested blood in the midgut (Akov 1965). However, the rate of digestion of a subsequent blood meal is normal. Similar results are produced with *A. aegypti* (1) fed metepa, apholate, or tepa for 2 days prior to a blood meal, (2) held in contact with a metepa treated glass surface or (3) irradiated with from 2,000 to 32,000 r (from a Co⁶⁰ source) (Akov, 1966). Irradiation of the mosquitoes does not influence the rate at which Evan's blue stained Dextran passes from the midgut. This indicates that the delay in emptying the midgut of a blood meal, by *A. aegypti* treated with irradiation or chemosterilants, is due not to an effect upon the gut mobility but upon the rate at which the digestion products are absorbed and utilized (Akov, 1966). In mosquitoes of the same age, the evacuation rate is lower during the second meal than during the first.

There are individual differences in the rate of digestion by *Anopheles punctulatus* Dönitz (MacKerras and Roberts, 1947). In this species about 25% of the females still have blood in the midgut 72 hours after feeding. MacKerras and Roberts suggested that if a large amount of blood is present at 48 hours the *Plasmodium* Marchiafava and Celli ookinetes may be imprisoned in this mass. The mean time for gut emptying is 48 hours for *A. gambiae* and 60 hours for *A. funestus* with considerable variation in the rate of digestion in both species (Hocking and MacInnes, 1949). In *A. gambiae* evacuation time varies from 24 to 72 hours; in *A. funestus* from 24 to 96 hours. *Anopheles maculipennis* whose ovaries are developing digest their blood meal in 73.4 to 87.1 hours, while parous females whose ovaries are not developing require 57.7 to 60 hours (Detinova, 1962). These findings led Detinova to suggest that neuro-hormonal regulation of digestion may occur in mosquitoes. There is a slight but significant decrease in the digestion rate with aging and Detinova (1962) reported that it takes longer to digest the first meal than to digest subsequent meals. This latter finding is the opposite of that reported for *A. aegypti* (Akov, 1966).

Digestion of blood is quite different in "long winged" and "short winged" *A. maculipennis* when they are overwintering (de Buck, Shoute, and Swellengrebel, 1932). For at least the first 24 hours, the blood ingested by the "long winged" females is divided into an anterior translucent half and a posterior, opaque, cellular mass. The erythrocytes show very little agglutination when the midgut is dissected in saline. At 24 to 26 C, digestion of the meal proceeds very slowly, requiring 5 or 6 (occasionally 10) days before the red colour (indicating undigested blood) disappears. In the "short-winged" females the sequence of events is quite different. Within half an hour of feeding the serum is absorbed from the lumen of the midgut leaving the cells in close contact with the gut epithelium. There is a marked agglutination of red cells and the digestion of the meal proceeds rapidly if the temperature is sufficiently high. If given more than one meal, a certain portion of the "long winged" population digests blood in the same manner as the "short-winged" females (de Buck, Torren, and Swellengrebel, 1933). The differences in digestion by "long-winged" and "short-winged" *A. maculipennis* are not observed during the summer.

Variations in digestion rate occur in *Anopheles claviger*. These are correlated with the season (Sella, 1920a) and with environmental temperature (Sella, 1920b). In *A. maculipennis* the rate of digestion of the blood meal slows down as the temperature falls in the autumn, (Guelmino, 1951). The unchanged condition of the red cells suggests that digestion and absorption in the fall are limited to water soluble materials that can be used to develop adipose tissue (Guelmino, 1951).

Detinova (1962 p. 57), reviewing some aspects of the digestive process in *A. maculipennis*, stated that "if the temperature rises to the optimum the speed of the processes increases, but at temperatures above the optimum they slow down".

Culiseta annulata taken from the Poole area of Dorset, southern England require 4 weeks to digest a blood meal "at temperatures experienced in November - February" (Service, 1968b). Other species (*A. claviger*, *C. annulata*, *Mansonia richiardii*, *Aedes dorsalis* (Meigen), *Aedes geniculatus* (Olivier), *Aedes detritus*, *Aedes punctor*, *Aedes cantans*, and *Aedes cinereus*) in this same area (Brownsea Island, Dorset) take 5 to 8 days to digest a meal of human blood during May to August or September (Service, 1968a).

Australasian anophelines complete digestion of a blood meal in 3 to 4 days under summer conditions (Roberts and O'Sullivan, 1949). Büttiker (1958) observed *Anopheles culicifacies* Giles and *Anopheles aconitus* Dönitz in several locations in southeast Asia which were in a quiescent state and whose midguts contained a blood meal which was "dark red, coagulated, very hard and almost completely desiccated."

The digestion of blood by a mosquito may be influenced by other materials fed to the mosquito. When various species of *Anopheles* are fed alternately upon gametocyte carriers and bananas, many mosquitoes die with undigested masses of blood in their midguts (Darling, 1910). Some *A. aegypti* fed upon CaCl_2 , MgCl_2 , or either of these plus oxytetracycline before and after ingesting blood from a chicken, still have a residue of blood in the midgut four days later when held at 26.5 C and 75% R.H., conditions under which digestion is normally complete in 3 days (Terzian, 1958). In mosquitoes with inhibited digestive processes these undigested residues are about 1/3 the size of the original clot, are orange-red, contain no intact red cells and give a positive heme test (benzidine reaction). (The positive benzidine test indicating the presence of intact heme groups is not surprising in view of O'Gower's (1956) finding that mosquito feces give a positive benzidine test). Inhibition of digestion can be prevented by adding the chelating agent ethylene diamine tetraacetic acid to the salt-antibiotic mixture. Since mosquitoes with a large residue of undigested blood are able to produce large numbers of eggs, Terzian (1958) concluded that it was "reasonable to assume that only digestion of the hemoglobin fraction, of all the fractions contained in the original blood meal, is affected by the cations, or cation antibiotic mixture." The validity of this assumption is questionable in view of the finding that the *in vitro* activity of *A. aegypti* proteinase upon both hemoglobin and serum albumin is inhibited by CaCl_2 , MgCl_2 , and MnCl_2 (Gooding, 1966a). Terzian (1963) expanded the *in vivo* inhibition studies to include both *A. aegypti* and *Anopheles quadrimaculatus*, 4 cations, and 4 antibiotics. Digestion of blood is inhibited to varying degrees in both species by the presence of calcium, magnesium, manganese or iron in sugar solutions consumed prior to the blood meal. The action of the antibiotics is very complex. Oxytetracycline by itself has very little effect on digestion by *A. aegypti* but potentiates the action of calcium, magnesium, and manganese. It suppresses inhibition by iron. However, oxytetracycline has a marked inhibitory effect on *A. quadrimaculatus* digestion, potentiates manganese only and inhibits the effect of iron. Penicillin reduces the effects of calcium and magnesium upon *A. aegypti*, but has itself an inhibitory effect on *A. quadrimaculatus* digestion. A mixture of chloramphenicol and dihydrostreptomycin in the diet of *C. pipiens quinquefasciatus* slows down

digestion of canary blood infected with *Plasmodium relictum* Grassi and Feletti to such an extent that about 1/3 of the mosquitoes still have blood in the midgut after 7 days (Micks and Ferguson, 1961). The results of these experiments with antibiotics are interesting in view of Arnal's (1950) claim that digestion of blood is initiated in *Culex pipiens* by bacteria which cause the haemolysis of the red blood cells.

Terzian and Stahler (1964) confirmed Terzian's (1963) findings and extended the observations as indicated below. *A. aegypti* completes digestion of a meal of chicken blood in about 72 hours at 26.7 C and 75% R.H. while *A. quadrimaculatus* required "at least 96 hours". Neither NaCl nor KCl at concentrations up to 0.3 M have any inhibitory effect upon digestion. Penicillin suppresses the inhibitory effects of oxytetracycline and neomycin in both species. Streptomycin inhibits digestion in *A. aegypti*; does not interact with oxytetracycline, but is suppressed by penicillin. Neomycin inhibits digestion by *A. aegypti* and *A. quadrimaculatus* and enhances the effects of calcium and magnesium markedly in the former but only slightly, if at all, in the latter. Neomycin enhances the inhibitory effect of manganese in both species. Iron suppresses the inhibitory effect of neomycin in *A. aegypti* but the inhibition by iron and neomycin are additive in *A. quadrimaculatus*. The inhibitory effects of the cations upon digestion in these mosquitoes led Terzian and Stahler (1964) to conclude that "the process of blood digestion is fundamentally the same in both species". However they suggested that the effects of the antibiotics upon a given physiological process vary with the species. Apparently these mosquitoes are able to produce viable eggs despite the inhibition of the digestion (Terzian and Stahler, 1964). This led Terzian and Stahler to conclude that the "effective inhibitory compounds do not interfere with the digestion or absorption of the plasma fraction of the blood but rather interfere with some one phase of the digestion of hemoglobin". This is essentially the same conclusion reached earlier by Terzian (1958) although no critical experiments were done to see if digestion of the serum proteins had, in fact, taken place.

Usually only female mosquitoes bite, although biting males have also been recorded (reviewed by Bates, 1949, p. 79). Whether or not the blood ingested by males is digested may vary with the species. Chao and Wistreich (1959) referred to the results of unpublished experiments indicating that male *Culex tarsalis* Coquillett could not digest force-fed blood meals and died shortly after the experimental meal. However, Russell (1931) was able to induce *C. pipiens quinquefasciatus*, *A. aegypti* and *Anopheles ludlowae* males to take blood meals which were dispatched to the midgut. In one male *C. pipiens quinquefasciatus* a single oocyst of *Plasmodium cathemerium* Hartman developed. No mention was made of unduly high mortality among the males. Males of *A. aegypti* and *C. pipiens quinquefasciatus* force-fed repeatedly, digested these blood meals in the same time as did the females (MacGregor, 1931).

Twenty-one of 23 blood-fed *Leptoconops (Holoconops) bequaerti* (Kieffer) held at 29.4 C emptied their guts by 40 hours after the meal. At 36.7 C 1 midge of 9 had an empty gut at 24 hours and by 28 hours 4 of the 9 midges had empty guts (Linley, 1965). Service (1968c) estimated the time for digestion of human blood by two species of midges (*Culicoides impunctatus* Goetghebuer and *Culicoides obsoletus* (Meigen) under field conditions in Dorset (southern England). During April (mean temperature 8.9 C) *C. obsoletus* took an average of 8.09 ± 0.63 days to digest its meal while in May (mean temperature 11.6 C) and June (mean temperature 15.9 C) the times were 5.79 ± 0.31 and 5.14 ± 0.39 days respectively. For *C. impunctatus* the time in June was 5.15 ± 0.33 days, in July (15.6 C) 5.27 ± 0.66 days; August (15.4 C) 5.09 ± 0.22 days, in September (15.8 C) 7.38 ± 1.05 days.

Stomoxys calcitrans which are hungry at the time they are offered blood will gorge until the abdomen is "not only more than twice its usual depth, but is also about half as broad

again as the normal breadth" (Hewitt, 1914). Within half an hour the abdomen may return to its normal size and by 2 hours the red color of the gut contents will no longer be visible externally. Brown feces are first passed about 6 hours after a blood meal and these were interpreted by Hewitt as being excretion of digested blood. Defecation of brown material usually ends about 72 hours after the blood meal and this time was interpreted as the period necessary for digestion of the blood meal. Digestion time varies from 50 to 95 hours and depends in part upon the size of the meal ingested. From the number and size of fecal deposits Hewitt concluded that digestion is most rapid 26 to 52 hours after feeding. Bishop (1913) reported that the rate of digestion by *S. calcitrans* is affected by weather. On the basis of the gross appearance of the abdomen at 24 hours after a blood meal 5 of 8, at 46 hours 9 of 10, and at 70 hours 8 of 8 wild caught male *S. calcitrans* had digested their meals (Anderson and Tempelis, 1970). The corresponding figures for females were 0 of 20, 5 of 20, and 7 of 24. Anderson and Tempelis (1970) summarized previous reports of the digestive rate of *S. calcitrans* and concluded that "the host source of the blood meal and the temperature at which flies are held both also affect digestion rates". They also reported (but without presenting the data) that with nulliparous *S. calcitrans* "the time elapsing between ingestion and digestion varies according to which state of a gonotrophic cycle she is in".

Vanderplank (1947), stated that with *Glossina swynnertoni* Austen age can probably affect the duration of the hunger-cycle and "young flies in the laboratory take smaller meals and digest them quickly".

Small clear globules of unknown composition appear in the ventriculus of *Diamanus montanus* (Baker) and *Xenopsylla cheopis* (Rothschild) (but not *Polygenis gwyni* (Fox), within an hour of a blood meal but usually disappear within a day (Holenreid, 1952). Unfed *P. gwyni* (but not *D. montanus* or *X. cheopis*) have bubbles in the ventriculus which disappear 1 to 24 hours after a blood meal. The flea ventriculus is swollen and bright red just after a blood meal but shrinks and darkens as digestion proceeds and in *D. montanus* is devoid of blood residue 1 to 10 days after the meal. None of these species defecate undigested blood. Parker (1958) observed that fleas (*Cediopsylla inaequalis inaequalis* (Baker), *Thrassis bacchi gladiolis* Jordan and *Pulex irritans* L.) fed on a variety of small mammals infected with *Pasteurella tularensis* (McCoy & Chapin) generally clear their digestive tracts of the bulk of the blood meal by 36 hours.

Nuttall (1917b) reported that the rate of digestion by *P. humanus* is influenced by temperature. The ingested blood remains red and the abdomen swollen for 4 days when the insects are kept at 12°C. At 31-37°C the size of the abdominal contents decreases rapidly in a few hours and as the blood is digested it turns from red to reddish-brown and finally to black.

HISTOLOGICAL CHANGES IN THE GUT AND BLOOD MEAL

Secretory and absorptive cells are not differentiated in the midgut of the hog louse, *Haematopinus suis* (L.) and these activities apparently are carried out by all gut cells (Florence, 1921). Within an hour of feeding, erythrocytes are vacuolated but leucocytes and platelets are not. Platelets are destroyed after 2 hours. The staining properties of leucocyte cytoplasm is slightly affected within 2 hours of feeding and by 6 hours after the meal the nuclei begin to disintegrate. By 8 hours the blood is an amorphous mass.

Pediculus humanus humanus L. (= *Pediculus humanus corporis* de Geer) at 30-32°C completely destroy the erythrocytes within 4 hours of feeding on humans (Cabasso, 1947). However, when lice are fed on guinea pigs the erythrocytes remain intact for 48 hours, about which time the lice die, many with ruptured guts. The mortality rates of unfed lice and

those fed human or guinea pig blood indicate that those fed guinea pig blood starve to death. Lice consuming a single meal on guinea pigs and subsequently feeding on man live a normal life span. Cabasso concluded that *P. humanus* can not digest guinea pig blood. Kryński, Kuchta and Becla (1952) claimed that guinea pig erythrocytes rapidly haemolyzed within the gut of *P. humanus*. Using a hanging-drop technique they showed that the haemoglobin crystallized in "trigonal pyramids of various size". Crystal formation began during the feeding period and within 6 hours the gut was filled with crystals which mechanically damaged the midgut epithelium. Guinea pig haemoglobin also crystallized in the gut of *Cimex lectularius* and *Ornithodoros moubata* (Murray) but in these species the crystals decomposed without injuring the gut wall (Kryński et al, 1952).

Davies and Hansens (1945) proposed the hypothesis that "the digestive enzymes of the louse were immunologically specific and developed to act upon the blood taken by the young insect in its first meal". This hypothesis was tested by rearing *P. humanus* for 10 days on either a man or a rabbit and then transferring half of each group to another man or another rabbit. Mortality during the next 9 days was independent of whether the lice had switched host species and thus the data did not support the hypothesis.

The rate at which mouse erythrocytes and *Spirochaeta duttoni* (Novy and Knapp) are destroyed in the gut of *C. lectularius* depends upon temperature. At 12, 14, 16, 20 and 24 C erythrocytes are still intact at 278, 122 (sic), 42, 31, and 8-24 hours after ingestion of the blood meal (Nuttall, 1908).

Erythrocytes from a sickle-cell anemia patient all exhibited the sickling form after 24 hours in the midgut of *Panstrongylus megistus* compared with only 1/3 "sickle-form" in a sealed control (Pick, 1955). Haemolysis began in 3 days and was complete by 6 days after feeding. By 15 days crystals of sickle-cell haemoglobin were observed. Crystals of normal hemoglobin were never observed in the gut of *P. megistus*.

Blood meals are stored in the expanded, anterior portion of the midgut ('stomach') of *Rhodnius prolixus* (Wigglesworth, 1936). Here the blood cells remain intact for several days and the haemoglobin red for several weeks indicating that no digestion is taking place (Wigglesworth, 1936). In the narrow, posterior portion of the midgut ('intestine') the blood turns dark brown or black indicating that this is the site of digestion.

Digestion of the blood meal by mosquitoes begins at the outer edge of the meal and proceeds inward (Davies and Philip, 1931). This occurs in *Culex pipiens* (Huff, 1934), *Aedes aegypti* (Stohler, 1957; Howard, 1962; Akov, 1965; Freyvogel and Staubli, 1965; Gander, 1968), *Anopheles stephensi*, *Anopheles gambiae* and *Anopheles labranchiae atroparvus* (reported as *A. maculipennis atroparvus*) (Freyvogel and Staubli, 1965).

Before engorgement the midgut epithelial cells of *A. aegypti* are columnar; during engorgement they become squamous with convex internal borders (Howard, 1962). As digestion proceeds, the cells return to their original shape. These observations were confirmed by Freyvogel and Staubli (1965) and were extended to *A. stephensi*, *A. gambiae* and *A. labranchiae atroparvus* (Staubli, Freyvogel and Suter, 1966). Although the shape of the epithelial cells changes in a reversible manner the shape of the tracheoles serving the midgut cells is irreversibly changed from a tight spiral before the first meal to slightly curved after the meal (Detinova, 1962).

A whorled granular endoplasmic reticulum, is present near each nucleus in the midgut cells of fasting or sugar-fed *A. aegypti*. During the ingestion of blood (by *A. aegypti* and *Aedes togoi* (Theobald), these whorls unfold. The whorls reform upon completion of digestion, and the endoplasmic reticulum may be involved in the secretion and transport of proteolytic enzymes (Bertram and Bird, 1961). These changes in *A. aegypti* were confirmed by Staubli, et al (1966). In unfed *A. labranchiae atroparvus*, *A. gambiae* and *A. stephensi* the

whorls found in *A. aegypti* are replaced by apical granules which disappear at the time secretions are detectable in the midgut lumen. The large globules of RNA-containing material found between the nuclei and the lumen borders of midgut epithelial cells of unfed *A. aegypti* by Dasgupta and Ray (1955) may be the whorls of endoplasmic reticulum reported by the workers cited above.

Staubli et al (1966) suggested four possible functions for the whorled endoplasmic reticulum. First, the midgut secretions (e.g. digestive enzymes) could be synthesized and stored in the whorls prior to ingestion of the blood meal, although the appearance of the whorls was not consistent with this. Second, immediately after feeding the whorls could rapidly synthesize the secreted material. Since the whorls break down into vesicles (Staubli et al, 1966) within 9 minutes of feeding it was suggested that synthesis was completed by this time. Third, synthesis of the secretory material could take place on the vesicles which arise from the whorls and fourth, the endoplasmic whorls could be concerned rather, with the absorption process. The first and second and, possibly, the second and third suggestions could be distinguished from each other by experiment. However, as far as I know the critical experiments on mosquitoes have not been done.

In *Anopheles maculipennis* the anterior midgut cells secrete a fairly large amount of a mucous-like material within 7 minutes of a blood meal. Although this mucous forms a plug at both ends of the stomach and often completely surrounds the meal the mucous "does not appear to exert any important effect on digestion, as the erythrocytes in its vicinity are hardly broken down at all" (Freyvogel and Staubli, 1965). Other species of *Anopheles* appear to produce a smaller quantity of mucus a little later than *A. maculipennis*.

In mouse-fed *A. aegypti* the midgut epithelium produces a granular secretion for up to 15 hours after feeding and the peritrophic membrane (PM) is apparently formed from this (Bertram and Bird, 1961). However, with chicken-fed *A. aegypti*, secretions in the form of discrete hemispherical droplets on the internal border of the cells first appear about 12 hours after engorgement (Howard, 1962). These droplets increase to a maximum size at about 40 hours, after which each appears to be attached to a cell by a stalk. In contrast Dasgupta and Ray (1955) observed in blood fed *A. aegypti* a holocrine secretion which disintegrated when discharged into the gut lumen. Howard (1962) interpreted the droplets he observed in *A. aegypti* as the substance from which the PM forms. The PM first appears about 12 hours after the mosquitoes feed. This membrane increases in size until about 36 hours, and by 48 hours is rather brittle. As the amount of blood in the midgut decreases the PM is fragmented by the contraction of the midgut muscles. In *A. aegypti* the PM forms several hours after ingestion of the blood meal but before digestion begins (Stohler, 1957). As digestion proceeds the PM becomes harder and more brittle but subsequently softens and as the meal is digested the PM adheres to the blood meal, not the midgut cells.

Yaguzhinskaya (1940) demonstrated the presence of a chitin-containing PM in blood-fed *A. maculipennis*. The membrane forms after a blood meal and is occasionally open at the posterior end. The remnants of the membrane are defecated after the meal is digested.

A chitin containing PM forms around the blood meal in the mosquito midgut (Waterhouse, 1953a). If, after partial digestion of the first blood meal, a second meal is taken, the second meal surrounds the first and a second peritrophic membrane is formed around the entire mass of blood.

The development of the PM has been studied in *A. aegypti*, *A. stephensi*, *A. labranchiae atroparvus*, and *A. gambiae* (Freyvogel and Staubli, 1965). In the first two species age and number of blood meals have no effect on the development of the PM. However, some specimens do not develop a complete PM and in these digestion is usually abnormal. In *A. aegypti* and *A. stephensi* feeding upon man, guinea pigs, rabbits or chicken the source of the

blood meal does not affect the formation of the PM. *A. gambiae* formed a PM but *A. labranchiae atroparvus* produce nothing more than a viscous material surrounding the blood meal. However, unlike the *A. stephensi* which lack a PM, *A. labranchiae atroparvus* digest the blood meal in a normal manner. In *A. aegypti* the PM forms in 5 to 8 hours after the meal and remains until digestion is practically complete (48 hrs). In *A. gambiae* PM formation requires at least 13 hours but may persist up to 60 hrs. The corresponding times for *A. stephensi* are 32 and 72 hours. The membrane in *Aedes* spp. passes through stages described as viscous, elastic, solid and finally fragile, but in anophelines it never develops beyond a delicate membrane.

Three species (*A. aegypti*, *A. gambiae*, *A. stephensi*) which normally form a PM do not do so completely if they ingest only a small quantity of blood (Freyvogel and Staubli, 1965). Mosquitoes feeding upon a chicken injected with heparin, or upon defibrinated blood, form a PM. *A. aegypti* and *A. gambiae*, feeding upon guinea-pig serum, form the membrane but *A. stephensi* usually do not. When *A. aegypti* are given an incomplete meal on guinea pigs, followed after 10 hours by a meal on chickens, they form a PM around each meal. The PM around the anterior (chicken blood) meal is thinner than around the posterior meal.

Ringer's solution will not dissolve the *A. aegypti* PM but does dissolve those of *A. gambiae* and *A. stephensi* (Freyvogel and Staubli, 1965). However Van Wisselingh's chitosan-iodine test is positive for PM of all three species.

A. aegypti consuming less than 0.1 mg of guinea-pig blood form no PM and they must ingest at least 0.5 mg before forming a complete PM (Freyvogel and Jaquet, 1965). However, there is no correlation between blood meal size and the condition of the PM in *A. stephensi* and probably not in *A. gambiae*. Both *A. aegypti* and *A. gambiae* produce a PM when they are given an enema of physiological saline or air. The PM formed in both species after a meal of blood or serum reacts positively to Van Wisselingh's chitosan-iodine test. However Freyvogel and Jaquet reported that the results of this test on the PM formed after a saline or air enema were inconclusive.

Formation of the PM and digestion of the blood meal in *A. aegypti* and *A. stephensi* have been studied in frozen sections by Gander (1968). The PM of these species have different structural features: in *A. aegypti* it is laminar while in *A. stephensi* it consists of a granular material imbedded in a Periodic acid-Schiff (PAS-) positive substance. In their initial stages of formation the PM of both species contain Periodic acid-Schiff positive material but during blood digestion this material disappears completely from the *A. aegypti* PM and partially from that of *A. stephensi*. Histochemical tests demonstrate the presence of carbohydrates and lipids in both PM's. In *A. aegypti* cells throughout the midgut epithelium undergo an apocrine secretion while in *A. stephensi* there is a modified merocrine secretion proceeding from the posterior to the anterior end of the midgut. Gander (1968) divided blood meal digestion by *A. aegypti* and *A. stephensi* into 2 phases. Early in phase I the midgut secretes carbohydrates and lipids. In the first 10 hours after a blood meal, carbohydrates are not detectable in the epithelial cells of *A. aegypti* but can be found in those of *A. stephensi*. Enzymes are probably secreted also during this phase but only erythrocytes at the very edge of the meal show signs of breakdown. Phase I ends when the PMs form, 16 hours in *A. aegypti* and 30 hours in *A. stephensi* after feeding, and no further secretion by the midgut epithelial cells occurs. Formation of lipid droplets within the blood meal and accumulation of these on the lumen side of the PM marks the beginning of Phase II. Digestion of the blood meal proceeds inward from the periphery and the epithelial cells accumulate carbohydrates and lipids. In *A. aegypti* the peak of lipid absorption occurs before the peak of carbohydrate absorption while the reverse is true for *A. stephensi*. Peroxidases occur in the midgut epithelial cells of both species of mosquitoes during digestion of blood; their concen-

tration remains constant in *A. stephensi* but in *A. aegypti* reaches a maximum 40 hours after feeding. Gander felt that there was a discrepancy between his observation that digestion proper did not begin in *A. aegypti* until about 16 hours after feeding, and the results of Fisk and Shambaugh (1952) and Gooding (1966b) which showed considerable proteinase in the gut by this time. He suggested that this discrepancy was connected with the presence of trypsin inhibitors within the blood meal.

Digestion of blood by *C. pipiens* was studied by deBoissezon (1930a, 1930b), Huff (1927, 1934) and Arnal (1950) using histological techniques. Huff's observations on *C. pipiens* are similar to those made on *A. aegypti*. The rate of digestion depends upon the amount of blood ingested and upon ambient temperature (deBoissezon, 1930a, 1930b). Hemolysis of the erythrocytes is followed by crystallization of their hemoglobin. The hemoglobin crystals are dissolved by the digestive juices and absorbed and digested by cells in the floor of the wide part of the midgut. Cells in the anterior, narrow part of the midgut produce a vitreous secretion from the nucleolar region and a granular secretion from their cytoplasm. Cells in the wide part of the midgut secrete vesicles which occasionally included their nucleolei. In *C. pipiens* Arnal (1950) observed merocrine secretion in two regions of the midgut during fasting and three types of secretion after a blood meal. He concluded that digestion of blood was initiated by symbiotic bacteria which penetrated the blood meal, caused the red cells, but not the leucocytes, to swell and eventually to lyse and which also prevented clotting. Simultaneously, the midgut cells began secreting. The cells in the narrow, anterior region released granules, those at the beginning of the wide portion of the midgut released vacuoles and holocrine secretion occurred in the cells in the floor of the widest part of the midgut. Arnal stated that the pH during digestion was 6.5 to 7, and speculated that the secretions observed were trypsins capable of acting in a slightly acid medium. Stroma and the leucocytes resisted digestion, but the bacteria apparently did not as they disappeared. Iron was detected in the young cells in the floor of the midgut with the Liesegang technique. As absorption took place, the midgut contents thickened, and the haemoglobin crystallized in the midgut lumen. Haemoglobin could not be detected in the hindgut.

In *Culicoides nubeculosus* the midgut epithelium of the unfed midge has columnar cells which become more or less cuboidal on ingestion of a blood meal (Megahed, 1956). A PM, not present in the unfed insect, forms within 5 hours of feeding on blood. The membrane varies in thickness and appearance in different parts of the stomach, having villi-like structures in some regions and a laminar appearance in others. The PM is apparently secreted by the midgut epithelial cells. By 24 hours, the PM develops a perforation at its posterior end through which material may pass from the midgut to the hindgut. The PM completely surrounds the blood meal except at its posterior end but a partial disintegration of one or more layers is evident in some regions. By 48 hours the blood meal is almost completely digested but the gut still contains haematin and some evidence of the PM. After 72 hours the midgut is empty of both blood residues and the remains of the PM.

During the first 2 days after *Culicoides obsoletus* feed on human blood the gut contents solidify and become opaque but change little in volume (Jamnback, 1961). Undigested blood and small black pigment granules and rods, presumably digestion products, occur in the gut by the third day. By the fourth day the blood meal is completely digested and the gut is empty when the midges are held at 21 C.

Feng (1951) examined the formation of the PM in *Phlebotomus mongolensis* Sinton and *Phlebotomus chinensis* Newstead fed on Chinese hamsters and in *Sergentomyia squamirostris* (Newstead) (reported as *Phlebotomus squamirostris*) fed on a toad. He studied also the influence of the PM upon establishment of trypanosomes in these sandflies. In *P. mongolensis* the PM completely envelopes the blood meal and is very tough. As in mosquitoes, digestion of

the blood meal begins at the periphery and progresses inward. Digestion of the blood meal requires 5-6 days and as material is digested and absorbed, the PM shrinks to a small spindle which is passed complete into the hindgut. *Leishmania donovani* Laveran and Mesnil flagellates live only within this peritrophic sac and pass into the hindgut within it. In *P. chinensis* a PM is formed but begins to break down 3 days after feeding, fragments of it passing into the hindgut with the blood meal residue. Digestion of a blood meal in *P. chinensis* takes about 7 days. The disintegration of the PM releases the flagellates which move forward and establish themselves in the proventriculus. Ultimately they migrate forward to the mouth parts. The PM of *S. squamirostris* appears to be open at the posterior end. Digestion of a blood meal by this species is complete within 3 days. Crithidia of *Trypanosoma bocagei* França leave the midgut through the open posterior end of the PM and establish themselves in the hindgut.

In *Phlebotomus papatasi* digestion is very slow and haemolysis of the erythrocytes takes place 3 or 4 days after feeding (Adler and Theodor, 1926). "Unaltered haemoglobin is never found in the epithelial cells of the stomach but it is passed in the feces" and it was concluded that it is the plasma which is the essential component of blood and not the erythrocytes. A PM is present a day or two after a blood meal.

Peritrophic membranes occur in *Simulium anatinum* Wood, *Simulium rugglesi*, *Simulium aureum*, *Simulium latipes*, *Simulium quebecense*, *Simulium croxtoni*, *Simulium venustum*, *Prosimulium decemarticulatum*, *Prosimulium hirtipes* (Fries) and *Cnephia ornithophilia* Davies, Peterson and Wood, (Bennett and Fallis, unpublished work cited by Fallis, 1964); *Simulium griseicolle* Becker, and *Simulium damnosum* (Lewis, 1950), and *Simulium neavei* Roubaud (Lewis, 1960). In *S. damnosum* the PM gives a positive chitosan test (Lewis, 1950, 1953) and is formed after ingestion of blood but not sugar (Lewis, 1953). Flies interrupted during feeding have blood in both the tubular (anterior) portion and the expanded (posterior) portion of the midgut. Engorged flies have all the blood in the posterior part of the midgut. During consumption of a blood meal some of the contents of the crop apparently pass into the anterior part of the midgut. A delicate membrane forms within half a minute of completion of engorgement and this membrane is quite distinct by 30 minutes after feeding. By an hour after engorgement the laminar nature of the PM is evident, particularly in the knob of the membrane at the posterior end of the midgut. The membrane gradually turns yellow and then brown. Between 24 and 72 hours after the meal, the blood mass decreases in size and the PM breaks up (Lewis, 1953). In blackflies, digestion proceeds from the periphery toward the centre of the blood meal (Fallis, 1964, citing unpublished work of Bennett and Fallis). Cells at the centre of the blood mass may remain intact for more than 48 hours.

"Resting" cells are columnar in the 'stomach' portion of the midgut of *Tabanus albimediis* (and other *Tabanus* spp.) but are converted to flattened pavement epithelium when the midgut fills with blood (Cragg, 1920). Secretory cells casting off large droplets are seen in the midgut most frequently during the 5 minutes after feeding and are rarely found more than 1 hour after a meal. The digestive substances acting on the erythrocytes cause the formation of dark pigments, beginning at the surface of the blood meal. One day after a blood meal and later, the epithelial cells become columnar again and secrete minute droplets of undetermined fate. Cells in the anterior portion of the midgut (cardia) secrete continuously. Columnar cells reform as digestion of the meal proceeds. There is no PM. Red blood cells are normal for a short time after ingestion but soon become distorted, shrunken, and poorly stained. Stroma are detectable for 24 hours in the gut. Pigments form early and by 2 hours the stomach contents are a purple, tarry mass. About 8 hours after feeding residue from the meal begins to pass into the hindgut.

No PM forms in the gut of *Chrysops silacea* (Wigglesworth, 1931; Crewe, 1961) and observations of the gut histology of this species made at various times after a blood meal "agree exactly with those of Cragg (1920) on *Tabanus*" (Wigglesworth, 1931).

The midguts of *Glossina palpalis*, *Glossina submorsitans* and *Glossina tachinoides* can each be divided into 3 sections on a histological basis (Wigglesworth, 1929). In all species the anterior half has an irregular columnar epithelium and includes a narrow band of giant cells containing bacteroids. In this region the blood is concentrated by removal of water but there is no digestion of the blood components. In the next region of the midgut there are large, deeply staining cells which, during digestion of blood, produce and release large vacuolated buds of cytoplasm from their apical surfaces; these buds later disintegrate in the lumen of the midgut. Blood in this region turns dark and becomes amorphous. The posterior region of the midgut has regular, columnar epithelial cells which become vacuolated late in the digestive process - probably indicating a role for them in absorption. A PM surrounds the blood meal and it is secreted by cells of the proventriculus. Hoare (1931) confirmed in *G. palpalis*, the existence of a PM consisting of a continuous, open-ended cylinder reaching from the proventriculus to the hind gut with new material being secreted at its anterior end each time a blood meal is consumed. Yorke, Murgatroyd and Hawking (1933) also reported PM's surrounding the blood meals of *Glossina morsitans* and *G. palpalis*. Weitz and Buxton (1953) cited unpublished observations of Jackson indicating that blood remains microscopically recognizable longer in laboratory held tsetse flies than in marked flies in the field.

The anterior part of the midgut of *Stomoxys calcitrans* consists of a blood reservoir with columnar epithelial cells (Lotmar, 1949). Although these cells secrete material (probably anticoagulins), the blood cells in a meal remain unchanged and no digestion takes place. Digestion proceeds as the blood moves posteriorly through the digestive region of the midgut and cyclic changes in merocrine secretion, absorption and cell regeneration occur. The formation of fat globules in isolated epithelial cells is observed 1 to 2 hours after ingestion of blood and these cells become more numerous as digestion proceeds. Digestion is more or less complete in 24 hours.

Minchin and Thomson (1915) described the histological changes occurring in the midgut of the flea *Nosopsyllus fasciatus* (Bosc) (reported as *Ceratophyllus fasciatus*) during digestion of *Trypanosoma lewisi* (Kent) infected blood meals. After the fleas feed, the midgut cells are flattened but become columnar as the meal is digested. Within a few hours of feeding the red blood cells break down and by 24 hours the blood meal is viscous and brick-red and contains large "grains". By 48 hours the stomach contents are watery and brownish-black and contain fewer smaller "grains". By use of an iron-haematoxylin-Lichtgrün-picric acid combination the stomachs of the fleas may be divided into 2 classes - a grey-black series with a greenish tinge and a bright lemon-yellow series. In the grey-black series there are many grains and spherules suspended in the greenish "coagulated albuminous matrix" by 18-24 hours after the meal. The centre of the gut contents lacks the coarse grains and is clear. The grains become smaller as digestion proceeds and by 36 hours only greenish-grey debris next to the epithelial cells remains. Leucocytes are recognizable 24 hrs, but not 36 hours after the blood meal. Minchin and Thomson concluded that "digestion, or more probably the passage backwards toward the rectum of the undigestible remnants, of the blood-debris appears to proceed from the center... towards the periphery". The stomachs of the yellow series contain a closely packed granular material and this and the matrix are stained by the picric acid. Digestion in this series is slower than in the grey-black series and Minchin and Thomson considered the yellowish stomachs to be abnormal.

The midgut epithelium of adult fleas (*Ctenophthalmus Kolenati* sp) has intranuclear crystals in about 10% of the cells (Richards and Richards, 1969). The existence of these crystals

in the blood-feeding adult but not in the scavenging larva led to the suggestion that they are derived from the hemoglobin of the blood meal.

Waterhouse (1953b) presented data on PM's based on a rather extensive survey of insect midguts. He classified the PM's as type I, if they consisted of 1 or more layers "produced mainly or entirely by a ring of cells at the anterior end of the midgut" and as type II, if they consisted "typically of a series of thinner, coaxial layers and arises by periodic delamination from the surface of the striated border of a layer of material secreted from the whole midgut epithelium". Type II PM's occur in adult mosquitoes (*A. aegypti*, *Culex pipiens quinquefasciatus*) and tabanids (*Dasybasis froggatti* (Ric.), *Scaptia jacksoniensis* (Guer.) and *Scaptia gattata* (Don) while type I PM's are found in the Nycteribiidae (*Nycterebosca falcozi* Jobl.) and some Hippoboscidae (*Ortholfersia macleayi* Leach and *Ornithomyia* Latreille sp but not *Melophagus ovinus* (L)).

SEROLOGICAL AND CHEMICAL ANALYSIS OF GUT CONTENTS DURING DIGESTION OF THE BLOOD MEAL

The precipitin technique has been used most frequently in host preference studies of mosquitoes but has also yielded data on digestion rates. Most of the latter demonstrate that after a certain length of time depending on environmental conditions, the midgut contents do not give a positive reaction (Bull and King, 1923; Davis and Philip, 1931; Weitz and Buxton, 1953; West, 1950). Bates (1949, p. 90) cites unpublished observations of Balfour on digestion rates in *Anopheles superpictus* Grassi, *Anopheles maculipennis* and *Anopheles sacharovi* Favre in Greece. The latter two species were similar and 100%, 91% and 79% of the mosquitoes gave positive tests after 2, 12, and 14 hours respectively. *A. superpictus* digested blood more rapidly and 96%, 72% and 39% gave positive reactions after 2, 12, and 14 hours.

Schubert and Kelley (1950) correlated the appearance of the blood meal with the precipitin reaction. *Aedes aegypti* were divided into three groups 17 hours after feeding on a bird (species not stated). Of the mosquitoes containing digested and haemolized blood 67% gave positive precipitin tests, 83% of the partially fed mosquitoes were positive and 96-100% of the fully engorged mosquitoes were positive.

West and Eligh (1952) studied the digestion rates in *A. aegypti* under laboratory conditions and in *Aedes hexodontus* under field conditions with the precipitin test. They showed that digestion in *A. aegypti* held in total darkness occurred more rapidly at higher temperatures (6 to 27 C). The rate of digestion of guinea-pig serum by *A. aegypti* has a $Q_{10} = 2.0$ in the temperature range 20 to 30 C (Williams, 1953). West and Eligh suggested that the rate of digestion could be influenced by light, and by the species of mosquito and host. It was pointed out that serological techniques indicate alteration of the blood meal proteins and not completion of digestion, and West and Eligh (1952) suggested that measurement of protease activity as done by Fisk (1950) "might give a more accurate indication of completion of blood digestion than would any other known method". In this respect the results of Akov (1965) are interesting. She found that in untreated *A. aegypti* as well as in those treated with 5-FU that there was a good correlation (coef. corr.= 0.872) between the amount of proteinase in the midgut and average stage of development of the ovaries in the mosquito. Confirmation of this was obtained by feeding mosquitoes citrated sheep blood containing 1.25 μg crystalline soybean trypsin inhibitor/2 mg blood; this inhibited both midgut proteinase and the development of the ovaries.

Weitz and Buxton (1953) ran precipitin tests at irregular intervals on mosquitoes kept at 25 C and 80% R.H. Two species, *Anopheles labranchiae atroparvus* and *A. aegypti*, fed on

man, were all positive after 16 hours and 9% and 27% were positive after 3 days. All were negative on the fourth and fifth day. All *Culex pipiens* (ssp) *molestus* Forskal fed on man, gave positive precipitin reactions 24 hours after the blood meal but all were negative on the third and fifth days. The percentage of ox-fed *Anopheles aquasalis* Curry giving a positive precipitin test also declined as digestion proceeded (95% after 16 hours, 26% at 20 hours, 4% at 30 hours, and 0% at 40 hours).

Differences in the rate of digestion of human blood by five species of mosquito were observed when they were held under identical conditions of temperature (27 C), photoperiod (ratio of light to dark was 1:1), and humidity (saturation deficit was 2 ± 1 mm Hg) (O'Gower, 1956). The time required for half of the mosquitoes to complete digestion to a point where the precipitin test was negative was 31 hours for *Aedes scutellarus* (Walker), 36 hours for *Aedes notoscriptus* (Skuse), 38 hours for *A. aegypti*, 46 hours for *Culex pipiens quinquefasciatus* and 48 hours for *Aedes australis* (Erichson). Since the intraspecific variation in the size of females was almost as great as the interspecific variation, O'Gower felt that "the different rates of digestion of human blood by the species of mosquitoes tested would seem to be due to specific differences in the digestive processes and not to specific difference in the size of the adults". O'Gower investigated the effect of photoperiod on *A. notoscriptus*. Mosquitoes were held under five conditions, ranging from continuous light to continuous dark and precipitin tests were run at 36 and 40 hours after the blood meal. As the ratio of dark to light increased so did the rate of digestion. O'Gower also stated (without supporting data) that one week old and three week old mosquitoes (species not stated) digested blood at the same rate.

Downe, Goring and West (1963) used the precipitin test to study the effect of both meal size and meal source on the rate of digestion by several species of mosquito. The time required for 50% or 100% of the mosquitoes to completely digest or denature human serum proteins were reported for *A. aegypti* and *Aedes trichurus* (Dyar). Using these criteria it appeared that females of both species given a small blood meal (i.e. where ratio of weight of blood ingested to weight of mosquito was less than one) digested the meal much more rapidly than those given a large blood meal. For *A. aegypti* the "50% digestion time" and the "100% digestion time" for small blood meals were 16 and 36 hours and for large blood meals were 40-44 hrs and 52-56 hours. The corresponding values for *A. trichurus* were 28 and 48 hours, and 64 and 76 hours.

The source of the blood meal (man, guinea pig, dog, or chicken) had little effect upon digestion rates in several species of *Aedes*, but did affect those of *Mansonia perturbans* (Downe, Goring, and West, 1963). In this species the "50% digestion time" and "100% digestion time" were 36 and 44 hours for chicken blood, 48 and 56 hours for guinea pig blood, 48 and 60 hrs for dog blood and 52 and 60 hours for human blood.

Templis and Lofy (1963) showed that *Culex tarsalis* digests blood meals from three different species of bird at three different rates. Positive reactions were obtained with mosquitoes fed on all three species of birds up to 18 hours after feeding, but at 24 and 36 hours the percentages were 70 and 29 for those fed on the white crown sparrow; 56 and 50 for those fed on the cow bird; and 100 and 73, for those fed on the English sparrow.

In *A. aegypti* given a small meal of human blood followed in 2 to 12 hours by a larger meal of guinea pig blood digestion of the human blood was prolonged (Downe, 1965). This probably occurred because the human blood meal was surrounded by the guinea pig blood meal and thus protected from digestion, which proceeded from the periphery toward the centre of the midgut contents.

Using agar double diffusion and immunoelectrophoretic analysis Mattern et al (1967) found that laboratory reared *C. pipiens quinquefasciatus* digested human albumin within 24

to 48 hours but retained the human immunoglobulin, IgG, in the midgut for 4 to 5 days. Wild caught mosquitoes fed on man and then kept in a tube for 4 days gave the same results. On the other hand, wild caught mosquitoes left free in a room for 48 hours after feeding on man were negative for IgG but gave strong positives for albumin. Even though a positive reaction for IgG was found in the midguts of caged *C. pipiens quinquefasciatus* for 4 or 5 days the appearance of a second precipitin band indicated that digestion of IgG began about 5 hours after the mosquitoes fed. Using the agar double diffusion technique these authors compared the IgG digestion products in the mosquito stomach with those produced by digestion of IgG with papain and trypsin. They concluded that "protein cleavage occurring in the stomach of mosquitoes is quite different from that produced by papain or trypsin". All these results were, however, based on a small number of mosquitoes.

Zaman and Chellappah (1967) studied digestion of human blood by *Armigeres subalbatus* using gel-diffusion and immunoelectrophoresis and concluded that the serum albumins persisted in the midgut longer than the serum globulins. The precipitin band for the former persisted for 48 but not 56 hours while for the latter it lasted 12 but not 18 hours. A similar pattern of digestion was obtained with *A. aegypti* digesting guinea-pig blood (Williams, 1953).

Herndon and Ringle (1967) used the double diffusion technique in microtubes to determine the length of time host antigens were detectable in the midgut of blood-fed *Anopheles quadrimaculatus* and *Culex pipiens*. Refrigerated (6-9 C) *C. pipiens* had identifiable antigens in the midgut for 12 days while in *A. quadrimaculatus* the antigens remained identifiable for only 1 week. At 25-28 C the antigens were identifiable for only about 1 day.

With precipitin tests Edman (1970a) showed that 2 and 4 day old *A. aegypti* digested human blood at about the same rate (at 27 C and 70% R.H.) while mosquitoes 6, 8, and 10 days old digested it at a slower rate. There was no difference in the rate of digestion by 8 and 22 day old mosquitoes. With 6 day old mosquitoes digestion was slower in virgin than in mated females. Digestion by 10 day old parous females was slower than by 10 day old nulliparous females. However digestion rates of the second blood meal in 10 day old mosquitoes were the same as of the third blood meal in 18 day old mosquitoes. This last finding differs from Akov's (1966) for the rate of emptying of the midgut of *A. aegypti*. Using immunological techniques and antisera of high titre, Edman (1970b) found no consistent differences in the rate of digestion of human albumin, γ -globulin, and α -globulin by *A. aegypti*. Complete denaturation of the proteins occurred between 60 and 66 hours after ingestion of the meal.

Of the *Culicoides nubeculosus* fed on man, 80% gave positive precipitin reactions after 24 hours, but all were negative after 3 days (Weitz and Buxton, 1953). In the same study it was reported that laboratory reared *Glossina morsitans* fed on man, ox, sheep, or goat were all positive for 2 days and on the third day gave 100%, 100%, 75%, and 90% positive reactions respectively. In contrast wild caught *Glossina swynnertoni* estimated as having fed on mammals 3 days earlier, gave only 28% positives and those estimated as having fed four days earlier gave only 7% positives.

Downe (1957) used serological techniques to follow the digestion of horse and guinea-pig blood (actually the serum) by several species of black-flies (*Simulium venustum*, *Simulium vittatum* Zetterstedt, *Prosimulium hirtipes* and *Simulium parnassum* Malloch). The rates of digestion were similar in all these species and were not markedly affected by the source of the blood. At 19.4 - 21.1 C and 75 - 80% R.H. precipitin reactions were obtained in nearly 100% of the insects 24 hrs after feeding. This declined to about 74% by 32 hours, 40% by 40 hours, and to 3% by 48 hours, after which no positives were obtained. The digestion rate in insects maintained under field conditions was retarded at lower temperatures. By using the Meyer reduced phenolphthalein test Downe could detect blood in 5 out of 8 *S. venustum*

50 hours after they were fed on a horse but similar tests on 11 *S. venustum* made 60 hours after feeding were all negative. Downe (1957) stressed that the serological test indicated only when serum proteins were modified and not when the blood meal was actually digested.

Holstein (1948) studied methods of producing specific antisera and presented some data on the length of time after a blood meal at which positive precipitin reactions could be detected. For *Pediculus humanus* positive reactions were obtained with 1/10 dilutions of antisera up to 13 days, with 1/100 up to 10 days and with 1/1000 up to 2 days after the lice had fed on humans. However it is not clear whether the lice lived for the full 15 days of the experiment after the blood meal. For *Cimex lectularius* positive reactions were obtained (dilutions of sera given in brackets) for 36 days (1/10), 30 days (1/100), 20 days (1/1,000), 14 days (1/2,000), 11 days (1/5,000), 6 days (1/10,000), and 1 day (1/15,000). Evidence that *C. lectularius* digested human blood slowly was obtained by Weitz and Buxton (1953) who found 100% were positive after 5 days, 97% positive after 10 days, 40% positive after 20 days, and 22% positive after 30 days.

Positive precipitin tests for human blood could be obtained in *Phlebotomus argentipes* Annadale and Brunetti for up to 8 days after they had fed once on human and then on mouse blood (Lloyd and Napier, 1930). Although the number of sandflies tested at each time after ingestion of the meal was small, the results indicated that the rate of positive reactions was unaffected by the duration of the digestion period! This suggests to me that some component of human blood, with which the anti-serum was reacting, was not digested by these sandflies.

By using the precipitin test on male *Stomoxys calcitrans* fed an unknown volume of citrated human blood Anderson and Tempelis (1970) obtained the following frequencies of positives: 1 of 2 at 12 hours, 3 of 4 at 24 hours, and 3 of 4 at 30 hours. The corresponding values for females were: 2 of 2 at 12 hours, 4 of 5 at 24 hours, and 2 of 3 at 30 hours. In one experiment, the weight of material ingested was known: males consumed 6.9 mg and females 10.5 mg of citrated human blood. Precipitin tests on these gave the following frequencies of positive for males: 6 of 11 at 25 hrs, 5 of 9 at 30 hrs and 0 of 10 at 33 hours after feeding. The data for females were 10 of 10 at 25 hours, 5 of 5 at 30 hours and 9 of 10 at 33 hours.

By spectroscopic examination of the gut contents of *Rhodnius prolixus* a month or more after feeding on a rabbit, Wigglesworth (1943) found evidence of oxyhaemoglobin, methaemoglobin and traces of acid haematin and concluded that "even after storage for this length of time in the stomach [=anterior mid-gut] digestion of haemoglobin has scarcely begun". A similar examination of the "coiled intestine" [=posterior mid-gut] showed that oxyhaemoglobin occurs only in the region near the stomach whereas acid haematin exists throughout. The haemoglobin is rapidly digested. The black residue remaining in the rectum consists of free haematin. Globulin apparently is digested leaving the unchanged iron porphyrin which is excreted. Some intact haemoglobin is absorbed from the digestive tract, but this is apparently not due to excessive stretching of the stomach, since bugs given a partial meal also absorb haemoglobin. Some of the absorbed haemoglobin passes into the haemolymph and some is digested in the midgut cells to form a modified haem pigment and free iron.

Digestion in the 'intestine' of *Triatoma infestans* is reported to "follow the same lines as in *Rhodnius*" (Wigglesworth, 1943). In *C. lectularius* "Digestion in the lumen of the gut proceeds as in *Rhodnius* but no brown or green pigments can be seen in the epithelium of the stomach or the intestine" (Wigglesworth, 1943).

The human body louse, *P. humanus humanus* digests its blood meal in the midgut but does pass undigested haemoglobin in the feces as evidenced by the presence of methaemoglobin and oxyhaemoglobin in the excreta (Wigglesworth, 1943). A positive reaction with

benzidine occurs, both with mosquitoes (*A. aegypti*, *A. scutellaris*, *A. notoscriptus*, *A. australis* and *C. pipiens quinquefasciatus*) 90 hours after feeding upon human blood and with the material defecated by blood fed mosquitoes. Thus O'Gower (1956) concluded that the haem of the haemoglobin was not broken down in the mosquito. The flea *Nosopsyllus fasciatus* apparently passes undigested haemoglobin in the feces (Wigglesworth, 1943).

Wigglesworth (1943), using spectroscopic techniques, found no evidence for absorption of undigested haemoglobin in mosquitoes (*A. maculipennis* and *A. aegypti*) or the flea (*N. fasciatus*). However evidence was obtained for its absorption in *Rhodnius*, *Triatoma*, *Eutriatoma*, *Cimex* and *Pediculus*.

The hematin crystals in the feces of *P. humanus* fed on man are rhombic plates whereas those in feces of *P. humanus* fed on rabbits are smaller and cubic (Davis and Hansens, 1945). Whether this difference is attributable entirely to the source of the blood or to possible differences in the way *P. humanus* digests human and rabbit blood was not mentioned.

Gooding (1966b) determined the amount of water soluble protein in the midguts of *A. aegypti* and *C. pipiens quinquefasciatus* fed on chickens. Assuming that the decline in midgut protein content represents digestion of protein by the mosquitoes two criteria can be applied for comparing the rates of digestion: (1) the decrease in protein content in mg/midgut/time interval or (2) the time required for a certain percentage of the protein in the meal to be removed. Using the first criterion *C. pipiens quinquefasciatus* digests its meal faster than *A. aegypti*. With the second criterion the reverse is true. The time required for a 50% and a 90% decrease in the protein content of the midgut was approximately 18 and 40 hours respectively for *A. aegypti* and 23 and 48 hours for *C. pipiens quinquefasciatus*. (Briegel (1969) reported that half the protein in a blood meal disappears from the midgut of *Culex pipiens* within 24 hours of feeding and that digestion was completed by 72 to 96 hours). These experiments were done under essentially the same environmental conditions as those of O'Gower (1956) except that the mosquitoes were fed on chickens by Gooding, (1966b) and on humans by O'Gower, (1956). O'Gower found that 50% of the *A. aegypti* and of the *C. pipiens quinquefasciatus* gave negative precipitin tests by 38 and 46 hours respectively. Gooding estimated that 90% of the water soluble protein had been removed from the midguts of *A. aegypti* and *C. pipiens quinquefasciatus* by 40 and 48 hours respectively. O'Gower in fact studied the denaturation of serum proteins and Gooding the decline of total water soluble proteins in both serum and hemoglobin. Although the two studies were done with blood-meals from different sources the results were consistent since *in vitro* studies showed that these mosquitoes hydrolyzed serum proteins slower than hemoglobin (Gooding, 1966a).

During the digestion of guinea-pig blood by *A. aegypti* the ratio of protein nitrogen to total nitrogen in the midgut declines during the first 48 hours after feeding indicating digestion of the meal (Williams, 1953). However during this period there is no decrease in the total nitrogen content of the midgut indicating either that there is no absorption or that nitrogenous materials are being secreted into the midgut.

Fisk (1950) estimated the pH of the midguts of *A. aegypti* and *A. quadrimaculatus* with indicators. Stomachs of unfed mosquitoes of both species had a pH of 6.5 while those of *A. aegypti* fed on human blood had a pH of 7.3. With a microelectrode, Micks, deCaires, and Franco (1948) found the pH's of the stomachs of unfed *C. pipiens*, *C. pipiens quinquefasciatus*, *A. aegypti*, and *A. quadrimaculatus* to be 7.27, 7.43, 7.31, and 7.59. When these species were fed upon chicks the pH values of the stomachs were 7.52, 7.60, 7.67 and 7.75. Similar measurements of blood from the stomachs of *A. aegypti* fed on chickens gave values ranging from pH 7.47 to pH 7.90 (Bishop and McConnachie, 1956). Twenty-four of the 30 samples had pH values between 7.60 and 7.76 and there was no correlation between the duration of the digestion (up to 70 minutes) and the pH value. From these pH estimates, it

may be inferred that the proteinases responsible for digestion of the blood meal must function in a slightly alkaline medium. MacGregor (1931) reported that the midgut of *A. aegypti* and *C. pipiens quinquefasciatus* fed upon a solution (pH 7) of bacto-peptone and B.D.H. universal pH indicator had a pH of approximately 3 to 4. Roy (1937), examining dissected *Gasterophilus intestinalis* larvae with indicators, determined the pH of various parts of the alimentary canal to be: salivary glands pH 7.1, proventriculus pH 7, middle part of midgut pH 7.4 and hindgut pH 6.8. The pH of the midgut of blood fed *Glossina submorsitans* was about 6.6. The exact mechanism for controlling the gut pH and the contribution of the buffering capacity of the ingested blood to its control have not been elucidated, nor has the relationship between gut pH and the pH optimum of all the enzymes functioning in the gut.

DeFreitas and Campos (1961) studied the rate of elimination of Fe⁵⁹ by fifth instar and adult *T. infestans* and by first instar *Panstrongylus megistus* fed upon a chicken which had had Fe⁵⁹ incorporated into its haemoglobin. The results indicated the rate of digestion of haemoglobin by these bugs. There was little or no excretion of Fe⁵⁹ during the first six days after feeding on the radioactive blood. The time required for elimination of 50% of the Fe⁵⁹ was 40 days for 1st instar *P. megistus*, 31 days for 5th instar, and 16 days for adult *T. infestans*.

The major nitrogenous wastes in the feces of *G. morsitans* are uric acid, arginine, histidine, and hematin (Bursell, 1965). A reasonably close agreement existed between the quantities of these compounds excreted after the first hunger cycle and the amount which would theoretically be produced from a blood meal. The differences between these amounts during the first hunger cycle were accounted for by the development of the flight muscles during this period. Bursell (1965) also found a correlation between the amount of blood consumed and the amount of uric acid produced. Thus one can use the rate of excretion to estimate the rate of digestion.

Langley (1966b) used this technique to show that male *G. morsitans* digests chicken and lizard blood faster than mammalian blood. However, the rates for digestion of blood from rat, guinea-pig, sheep, cow, bushpig, or man do not differ significantly. Digestion of impala blood is also at about the same rate as that of other mammals (Langley, 1968a). Laboratory reared, non-teneral males feeding on guinea-pigs digest their meal more slowly than field-caught, non-teneral, males. Digestion is fastest in males which are caught after they have fed upon oxen (Langley, 1966b). These differences led Langley (1966b) to propose that the prefeeding behaviour of the flies affected the subsequent rate of digestion. Non-teneral, field-caught males feeding upon guinea-pigs digest this meal at the same rate when held in continuous light as when held in total darkness, even though the males are less active in total darkness.

Field-caught male and female *G. morsitans*, feeding on oxen, digest their blood meals more rapidly than laboratory reared males and females feeding on bovine or guinea-pig blood (Langley, 1967a). Laboratory-reared, fertilized females digest their blood meal more rapidly than unfertilized females. Male *G. morsitans* feeding on ox blood in the field, excrete their blood meal more rapidly than field-caught males feeding upon guinea-pigs in the laboratory (Langley, 1967c). There is no difference between rate of digestion of guinea-pig blood and cow blood (Langley, 1967b). Field-caught males fed several times on guinea pigs in the laboratory digest each meal more slowly than the preceding one until by the third meal the rate of digestion is only slightly greater than for laboratory-reared flies (Langley, 1966c). The digestion rate in male *G. morsitans* is not affected by the sex or reproductive condition of the guinea-pigs upon which they feed (Langley, 1968b).

DIGESTIVE ENZYMES AND THEIR PROPERTIES

A variety of digestive enzymes have been found in blood-sucking insects. Interpretation of the literature is straight forward except for the frequent occurrence of the word "trypsin" to describe a proteinase with maximum activity in an alkaline medium. There are several proteinases (carboxypeptidase, amino-peptidase, chymotrypsin, etc) which are not readily distinguished from trypsin on the basis of the pH-activity curve alone. Therefore, in this discussion, the term "trypsin" will be reserved for an alkaline proteinase which cleaves peptide bonds on the carboxyl side of a basic amino acid and "chymotrypsin" for one which cleaves on the carboxyl side of an aromatic amino acid. Such designations are based upon the use of synthetic substrates. Proteolytic enzymes active in the alkaline region, without adequate demonstration of the bond specificity, shall be referred to as proteinases or, if necessary, alkaline proteinases.

Two proteolytic enzymes occur in whole *Aedes aegypti* adults (Wagner, Tenorio and Terzian, 1961). One of the enzymes is a trypsin found in the midgut of the female but not in the rest of the mosquito nor in any part of the male. This enzyme separates into two fractions on a DEAE-cellulose column, the 2 fractions have similar properties. The other enzyme hydrolyzes denatured hemoglobin. It has maximum activity at pH 7.5, but functions almost as well up to pH 9. The purified enzyme is quite stable in acid, losing none of its activity at pH 3 when heated to 96 C for ten minutes. Wagner et al ran 2 experiments with females to determine whether the activity was localized in the midgut; in one all of the activity was in the gut; in the second 53% of the activity was in the gut homogenate. Thus, the authors concluded that this proteinase was primarily a digestive enzyme.

The tryptic & chymotryptic activities of *A. aegypti*, *Culex pipiens quinquefasciatus* and *Pediculus humanus* are due to 2 different enzymes and for each species the chymotrypsin has a higher molecular weight than the trypsin (Gooding, 1968, 1969). The major chymotrypsin fractions from the midguts of larval and adult *A. aegypti* are approximately the same molecular weight (Yang and Davies, 1971). Trypsin from adult *A. aegypti* has a molecular weight of 21,500 (Huang, 1971a). The proteinases in *Rhodnius prolixus* and *Cimex lectularius* have a high molecular weight ($\geq 160,000$) (Gooding, 1968, 1969).

On paper electrophoresis, using a barbital buffer at pH 8.0, 3 cationic bands of proteolytic activity were found in *Stomoxys calcitrans* midguts (Patterson and Fisk, 1958). However, using starch gel electrophoresis, cationic bands were never found but 3 bands of proteinase activity were found migrating toward the anode at pH 7.6 (tris-citrate buffer) and pH 8.0 (barbital buffer). By comparing different electrophoretic fractions (from the starch gel electrophoresis) with respect to the relative rates of hydrolysis of azocasein and azoalbumin Patterson and Fisk concluded that at least two "trypsin-like" enzymes existed in the midguts of *S. calcitrans*.

Crystallized hemoglobin is hydrolyzed almost as rapidly by *A. aegypti* and *C. pipiens quinquefasciatus* as denatured hemoglobin (Gooding, 1966a). However it is not known whether the crude midgut homogenates used in these experiments contained substances which denatured the hemoglobin. If no denaturing agents were present, then the proteinases of these mosquitoes may differ significantly from mammalian trypsins which do not readily attack native proteins (Sumner and Somers, 1947, p. 175). Denaturation of the proteins within the mosquito's midgut prior to digestion has not been demonstrated and Fisk (1950) wondered whether the proteinases of mosquitoes normally attacked native proteins or proteins denatured by some as yet unknown mechanism. He suggested that coagulation and agglutination of blood denature the proteins sufficiently to permit attack by mosquito trypsin.

With proteinases from *A. aegypti* and *C. pipiens quinquefasciatus* assayed at pH 7.9, the following are the K_m values (in mg/ml) for blood proteins: denatured hemoglobin, 1.51 and 1.32; crystallized hemoglobin, 1.84 and 3.15; bovine serum albumin fraction V, 19.3 and 8.51; and γ -globulin fraction II, 374, and 6.22 (Gooding, 1966a). At pH 9.5 there is little hydrolysis of the serum proteins by either species. The K_m values for *A. aegypti* and *C. pipiens quinquefasciatus* are: denatured hemoglobin 2.75 and 2.10, and crystallized hemoglobin 4.08 and 2.17. Using purified *A. aegypti* midgut trypsin, the K_m values at pH 7.9 are 2.24 mM for denatured hemoglobin and 0.47 mM for benzoyl-DL-arginine-p-nitroanilide (BAPNA) (Huang, 1971a). Davies and Yang (1968) reported trypsin with a pH optimum of 8.4 from the midguts of 6 simuliid species (*Cnephia dacotensis* (Duar and Shannan), *Prosimulium decemarticulatum*, *Prosimulium fuscum*, *Simulium rugglesi*, *Simulium venustum* and *Simulium vittatum*). The K_m values for tosyl-L-arginine methyl ester (TAME) are 2.4 mM for *S. venustum* and 3.1 mM for *S. rugglesi* (Yang and Davies, 1968b).

The *in vitro* temperature optima for *A. aegypti* and *C. pipiens quinquefasciatus* proteinases are in the range 46 to 50 C (Gooding, 1966a, 1968), that for *S. calcitrans* near 50 C (Patterson and Fisk, 1958), and for *C. lectularius* and *R. prolixus* about 45 to 50 C (Gooding, 1968). These optima are typical of alkaline proteinases. The temperature optima for mammalian trypsins range from 45 to 55 C (Buck, Bier, and Nord, 1962). For the housefly the optimum is 45 C (Lin and Richards, 1956), and for the larval blowfly it is 44 C (Evans, 1958). The *A. aegypti* proteinase experiments of Fisk (1950), Fisk and Shambaugh (1952), and Shambaugh (1954), were carried out at approximately 40 C, a temperature at which the enzyme is functioning at only half its maximum rate. Wagner et al (1961) carried out assays with *A. aegypti* proteinase at 30 C, a temperature at which the enzyme has about one quarter the activity it has at its temperature optimum.

The activity of the non-trypsin proteinase from *A. aegypti* is increased by diisopropyl-fluorophosphate (DFP), p-chloromercuribenzoate and sometimes cystine. Crystalline soybean trypsin inhibitor gives some inhibition, as do several cations (magnesium, calcium, mercury, and manganese) (Wagner et al, 1961). The alkaline proteinase activity of *A. aegypti* and *C. pipiens quinquefasciatus* is inhibited to some extent by calcium, magnesium, and manganese when denatured hemoglobin or bovine serum albumin are used as the substrates (Gooding, 1966a). However the proteinase from *S. calcitrans* is not affected by several ions (calcium, magnesium, sodium, chloride, or fluoride), penicillin G, or dialysis against distilled water (Patterson and Fisk, 1958).

Cations have varying effects upon partially purified *A. aegypti* trypsin (Wagner et al, 1961). Magnesium and manganese have no effect, but calcium, mercury, cadmium, and zinc inhibit the enzyme to varying degrees. The enzyme is inhibited by p-chloromercuribenzoate but cystine has no effect. This enzyme is not inhibited by crystalline soybean trypsin inhibitor (Wagner et al, 1961). However, Akov (1965) stated that soybean trypsin inhibitor inhibited *A. aegypti* trypsin *in vitro* and presented data to show that mosquitoes fed on citrated sheep blood containing 0.625 μ g/ml had less than half the proteinase activity found in the controls 24 hours after the meal. Gooding (1969) reported inhibition of trypsin from *A. aegypti* and *C. pipiens quinquefasciatus* and chymotrypsin from *P. humanus* by soybean trypsin inhibitor.

A. aegypti trypsin is inactivated by DFP (Wagner et al, 1961). Phenylmethane sulphonyl fluoride (PMSF) inhibits trypsin from *A. aegypti* and *C. pipiens quinquefasciatus* and chymotrypsin from *P. humanus* (Gooding, 1968, 1969). (These inhibitors are known to inhibit mammalian trypsin and chymotrypsin by reaction with serine at the active center of the enzyme; therefore, it may be inferred that the mosquito and louse enzymes studied have serine at their active centers.) Tosyl-L-lysine chloromethyl ketone (TLCK) inhibits *A. aegypti* and

C. pipiens quinquefasciatus trypsin but not *P. humanus* chymotrypsin, while tosyl-amide-phenylethylchloromethyl ketone (TPCK) inhibits *P. humanus* chymotrypsin but not *A. aegypti* or *C. pipiens quinquefasciatus* trypsin (Gooding 1968, 1969) (TLCK is a specific inhibitor of mammalian trypsin while TPCK is a specific inhibitor of mammalian chymotrypsin. Both compounds react with histidine at the active center and therefore it may be inferred that the insect enzymes studied have histidine at their active centers).

Although Fisk (1950) used heparinized whole rabbit blood as a substrate for most of his experiments he used a 1% solution of serum albumin for his studies on the effects of pH. Fisk calculated that during the assay only 0.34% of the available protein in the rabbit blood was hydrolyzed and concluded that the presence of 4 mg of blood per midgut was saturating the enzyme with substrate. He noted that the reaction rate when serum albumin was used was almost 3 times as great as when blood was used as the substrate. He suggested that blood from the rabbit may contain some substances which inhibit the midgut proteinase of *A. aegypti* and that some of the mosquito proteinase may combine with the inhibitor to neutralize it in the midgut. Gooding (1966a) demonstrated that serum from both normal and *Plasmodium gallinaceum* infected chicks will inhibit the *in vitro* activity of proteinases from *A. aegypti* and *C. pipiens quinquefasciatus* when denatured hemoglobin is used as the substrate. To obtain a 50% inhibition of the proteinases, from 1 to 7 μ l serum/ml reaction mixture was required.

The sera of 17 vertebrate species and the hemolymph of *Periplaneta americana* (L.) inhibit *A. aegypti* midgut trypsin (Huang, 1971a). The inhibition capacity of whole serum varies from a low of 0.01 μ g trypsin inhibited/ μ g serum for the dogfish to a high of 0.21 μ g trypsin inhibited/ μ l serum for the chicken. Huang used G-200 Sephadex gel filtration to determine the minimum number of inhibitors in serum and estimate their molecular weights. *P. americana* hemolymph has 1 inhibitor with a molecular weight of $\leq 11,000$. All of the vertebrate species examined have an inhibitor with a molecular weight between 31,800 and 66,100. This is the only inhibitor in 4 species (dogfish, turkey, chicken, and rat). All other vertebrate species have a second inhibitor with molecular weight $\geq 160,000$. A third inhibitor with a molecular weight between 77,600 and 107,000 occurs in 4 species (man; turtle, frog, and pike).

Huang (1971b) partially purified two inhibitors of *A. aegypti* trypsin from bovine serum. One of these, inhibitor I, is electrophoretically associated with the α_1 -globulin fraction of serum, has a molecular weight of about 43,500 and combines with trypsin in the molar ratio of 3.5 inhibitor I molecules to 1 trypsin molecule. Hill plots indicate that 2 molecules of inhibitor I inactivate 1 enzymic site of trypsin. Inhibition of trypsin by inhibitor I is competitive when haemoglobin is the substrate at 37 C. When BAPNA is the substrate inhibition is competitive at 30 and 34 C and non-competitive at 37 and 44.5 C. Inhibitor II is electrophoretically associated with α_2 -macroglobulin, has a molecular weight of $\geq 160,000$ (possibly as high as 1,000,000) and forms a complex with *A. aegypti* trypsin in the ratio 1.7 molecules inhibitor II/molecule of trypsin. Hill plots indicate that 2 molecules of inhibitor II inactivate 1 enzymatic site of trypsin. Huang demonstrated the complex of trypsin and inhibitor II electrophoretically and by gel filtration. This complex has very little proteolytic activity when hemoglobin is the substrate but retains most of the esterolytic activity when BAPNA is the substrate. Inhibitor II has the interesting property of protecting the mosquito trypsin against inhibition by inhibitor I, soybean trypsin inhibitor and PMSF but not against inactivation by TLCK. Although the pH optimum is similar for free trypsin and trypsin-inhibitor II complex, the K_m for BAPNA is lower for the trypsin-inhibitor II complex (0.21 ± 0.007 mM) than for free trypsin (0.47 ± 0.01 mM). When hemoglobin is used as a substrate, inhibitor II is a competitive inhibitor at 37 C but when BAPNA is used as the

substrate inhibition is competitive at 30 and 34 C and non-competitive at 37 and 44.5 C.

The chymotrypsin from *A. aegypti* larvae is inhibited by human and horse sera and in both cases inhibition is associated with the α -globulin fraction (Yang and Davies, 1971). Inhibition by human α -globulin is competitive. The inhibitors are unaffected by heating to 60 C but are inactivated at 100 C.

In view of the presence of a trypsin inhibitor in the hemolymph of *P. americana* (Huang, 1971a) it is interesting that the trypsin activity of the midgut of blackflies is approximately the same as homogenates of whole flies (Yang and Davies, 1968b). This latter observation led Yang and Davies to conclude that blackfly tissues probably do not contain materials inhibiting their trypsin.

The midguts of several insects have substances which influence the clotting of blood. The occurrence of coagulins and anticoagulins among various species of insects is summarized in table 2.

The anterior third of the midguts of *Glossina tachinoides* and *Glossina morsitans* contains an anticoagulin, but by removing the salivary glands it can be demonstrated that this anticoagulin is derived from the salivary glands (Lester and Lloyd, 1928). However, the anticoagulin found in the midgut of *Anopheles maculipennis* probably does not originate in the salivary glands, since it is destroyed by heating to 80 C while the salivary gland anticoagulin is stable at 100 C for 35 mins (de Buck, 1937).

R. prolixus midgut anticoagulin is stable at 60 and 80 C for 30 mins, is not affected by treatment with 0.1 N HCl or 0.1 N NaOH at R.T. or 60 C for 30 mins, but is destroyed by heating to 100 C for 5 mins (Hellmann and Hawkins, 1964). It is not precipitated by centrifuging at 100,000 g for 30 mins but is removed from solution by dialysis in the cold. It prevents clotting of rat, guinea-pig, cat, and human blood. The gut anticoagulin was designated Prolixin-G by Hellmann and Hawkins (1965). Prolixin-G inhibits thrombin and has a molecular weight between 100,000 and 200,000, is soluble in saline but is insoluble in water and is therefore possibly a euglobulin. It is less stable than the salivary anticoagulin, Prolixin-S (which also occurs in the midgut) when tested by storage at -20 C for 24 hours, freeze-drying or dialysis. It is, however, more stable than Prolixin-S when exposed to dilute trypsin solutions (Hellmann and Hawkins, 1965).

An antithrombin in the midguts of *Triatoma maculata* is distinct from the salivary anticoagulin (Hellmann and Hawkins, 1966). The gut anticoagulin is not affected by incubation with protamine sulfate, is fairly stable at 60 and 80 C, but loses about half its activity at 100 C in 2 mins and all its activity in 30 mins. Anticoagulin activity is not lost by treatment with 0.1 N HCl for 10 mins at R.T. or 60 C, but is lost in 0.1 N NaOH at 60 C in 10 mins. Freezing and freeze-drying cause a loss of activity but the anticoagulin is stable in the refrigerator. This antithrombin has a molecular weight between 100,000 and 200,000, is resistant to trypsin but loses some activity when dialyzed against saline (Hellmann and Hawkins, 1966).

Hellmann and Hawkins (1966) found no indication of fibrinolytic activity in the salivary glands of *T. maculata*. The gut, however, contains a plasminogen-activating factor but no active fibrinolytic enzymes. Guts with low anticoagulin activity have high fibrinolytic activity and visa versa. Freeze-drying decreases the fibrinolytic activity of the gut preparation but storage of dry preparations under refrigeration and storage at -20 C does not cause a loss of activity. The activity is not destroyed at 60 C but is in 2 mins at 80 C or 100 C and in 5 mins at R.T. in 0.1 N HCl or 0.1 N NaOH. The properties of the gut antithrombin from *T. maculata* are very similar to the gut antithrombin from *R. prolixus*. (Hellmann and Hawkins 1966).

The gut homogenates, but not the salivary gland homogenates, of *R. prolixus* contain

fibrinolytic activity. The gut contains a fibrinolytic activator and there is evidence that it also contains some weak fibrinolytic enzyme (Hellmann and Hawkins, 1964). The fibrinolytic activity in the gut is destroyed by heating to 100 C for 5 mins and by treating with 0.1 N NaOH or 0.1 N HCl for 30 mins at R.T. A 5 minute exposure to 0.1 N HCl causes a 70% loss of activity while 0.1 N NaOH has no effect in 5 mins. A single gut contains 1 N.I.H. unit of urokinase. A dialyzable fibrinolytic inhibitor is also present in the midguts of fed *R. prolixus*; the source of this inhibitor (insect or host) is unknown. The addition of soybean trypsin inhibitor to the *R. prolixus* gut extract does not inhibit the lysis of fibrin and it was concluded that "it is therefore unlikely that the fibrinolytic activity of the gut extract is due to trypsin".

Hawkins and Hellmann (1966) demonstrated that the midgut of *R. prolixus* contains a plasminogen activator which is detectable by measuring either fibrinolysis or caseinolysis and they proposed the name "rhokinase" for this material. Rhokinase activates plasminogen directly and its activity in the assay systems increases with time. The source of rhokinase in the midgut is unknown but it is not detectable in the blood of the guinea-pig on which the bugs fed, the salivary glands, the midgut cells of the bug, or in cultures of the bug's symbiont, *Nocardia rhodnii* (Erikson).

Lysis of the blood clot in the digestive tract of *Glossina austeni* is accomplished by two agents (Hawkins, 1966). The salivary glands and crop contain a substance presumably from the salivary glands, which activates plasminogen, resulting in lysis of the clot. The midgut and hindgut also contain an enzyme which is inhibited by soybean trypsin inhibitor. This enzyme was presumed by Hawkins (1966) to be trypsin. Hawkins showed that this same enzyme was responsible for clot formation of oxalated plasma.

A coagulin found in the abdomen (presumably in the midgut) of *Pthirus pubis* can neutralize the anticoagulin found in the head and thorax (presumably in the salivary glands) (Grusz, 1923). Similarly, the coagulins in the midguts of *Culiseta annulata* and *Culex pipiens* hasten clotting of both normal blood and that which was treated with the salivary glands of *A. maculipennis*, *C. pipiens quinquefasciatus*, or *C. annulata* (de Buck, 1937). Presumably, the coagulins neutralize the salivary anticoagulins. The coagulin from *C. annulata* is not destroyed by drying or by heating dry material to 99 C for 1 hour but saline solutions are inactivated by heating to 50 C for 15 mins.

The midgut coagulin from *G. morsitans* is completely inactivated by treating with 0.1 N KOH for 10 mins, or by heating to 80 C for 15 mins, while treating with 0.1 N HCl for 10 mins destroys about half the activity (Lester and Lloyd, 1928). By mixing salivary gland and midgut homogenates in various ratios and by adding these mixtures to sheep blood, the clotting time may be prolonged or shortened. If the salivary anticoagulin is added to blood 1 min before adding varying amounts of coagulin, the clotting time can be shortened, but not to the same extent as when the coagulin is added to the anticoagulin before mixing with the blood. Lester and Lloyd interpreted these findings as indicating that the midgut coagulin inactivates the salivary gland anticoagulin and they suggested that the midgut coagulin was similar to vertebrate kinase (i.e. the enzyme which converts prothrombin to thrombin). Lloyd (1928), speculating on the relationship between the function of the midgut coagulin and clot formation, stated that the "main function of this clot appears to be that it puts a brake on to the fluid meal and holds it in the proper region of the gut while digestion begins". The coagulin from *Musca crassirostris* is destroyed by heating to 100 C for 10 mins and its concentration in the midgut reaches a maximum 20 to 44 hours after a meal (Cornwall and Patton, 1914).

In invertases from *P. fuscum* and *S. venustum* have maximum activity at pH 6.2 (Yang and Davies, 1968c; Davies and Yang, 1968). The synthesis of oligosaccharides by the invert-

ase of *S. venustum* was detected by Yang and Davies but the products were not identified so it was not established whether the invertase was of the α -glucosidase or β -fructosidase type.

Gasterophilus intestinalis midgut amylase has optimal activity at pH 6 and is activated by chloride (Tatchell, 1958). Maltase and invertase have optimal activity at pH 6. Tatchell demonstrated that lipase hydrolysing tributyrin had maximal activity at pH 7 but no hydrolysis of olive oil or ethyl butyrate was demonstrated. The amylase from both male and female *S. venustum* has a pH optimum at approximately pH 6.5 (Yang and Davies, 1968a). The K_m (starch) is 0.65 mg/ml for female *S. venustum* amylase (Yang and Davies, 1968a).

ENZYME CONTENT OF THE GUT

Proteolytic activity in the midgut of *Aedes aegypti* is significantly higher in mosquitoes one or two hours after a partial blood meal than in sugar-fed mosquitoes (Fisk, 1950). The addition of homogenates of crops and/or salivary glands does not markedly increase the proteolytic activity of midgut homogenates of unfed mosquitoes. Immediately after *A. aegypti* feed on human blood, the proteinase activity in the midgut drops below the level of the unfed midgut. When mosquitoes are kept at 26.6 C and 50% R.H. the activity rises to a maximum about 18 hours after feeding and then slowly declines (Fisk and Shambaugh, 1954). Fisk and Shambaugh proposed that the initial decline in proteinase activity in the midgut was due either to depletion of enzyme due to an excess of substrate or to the presence of an antitrypsin in the serum. Feeding on sugar causes a slight increase in proteolytic activity after one hour, but the level returns to normal by two hours. Secretion of the proteolytic enzymes is primarily in response to serum proteins in the meal and there is a direct correlation between the amount of blood ingested and the proteinase activity of the midgut homogenate (Shambaugh, 1954). Incubation of *A. aegypti* midgut homogenates with blood for 18 hours at 40 C does not result in production of detectable quantities of proteinase, but midguts dissected from mosquitoes 18 hours after feeding on human blood have a large quantity of proteinase.

A. aegypti feeding on 5-fluorouracil (5-FU) in sugar solutions prior to, or with, the blood meal have lower midgut proteinase levels than controls 24 hours after the blood meal (Akov, 1965). The suppression of the proteinase level decreases with time when the mosquitoes are taken off the 5-FU diet. Mosquito proteinase is not inhibited *in vitro* by 5-FU.

A. aegypti treated with metepa (applied topically or fed a sugar solution), apholate (sugar solution) or gamma irradiation have a normal amount of midgut proteinase one day after feeding on a rat (Akov, 1966). The treated mosquitoes, however, retain blood in their midguts longer than the controls and two days after feeding have much more midgut proteinase than the controls (which, incidently, have empty midguts). When metepa is mixed with citrated sheep's blood and fed to *A. aegypti* through a membrane, the midgut proteinase activity is higher than in the controls but even so the blood meal is retained longer in the metepa treated insects.

Gooding (1966a) found much more proteinase in the midguts of *A. aegypti* and *Culex pipiens quinquefasciatus* 24 hours after feeding on chicks than in the midguts of unfed mosquitoes. In these experiments denatured hemoglobin was used as the substrate and the elevated proteinase levels in the fed mosquitoes were found at all pH values from 4 to 11. Proteinase activity in *C. pipiens quinquefasciatus* reached a maximum 36 hours after feeding and in *A. aegypti* usually 24 hours (but on 1 occasion 36 hours) after feeding (Gooding, 1966b). The time at which the maximum concentration of proteinase was present in the midgut was not influenced by holding the mosquitoes in continuous light or continuous darkness. In a single experiment in which *A. aegypti* and *C. pipiens quinquefasciatus* were

fed on normal or *Plasmodium gallinaceum* infected chicks, those mosquitoes which fed on the infected bird had a higher proteinase content in their midgut. Two experiments were run comparing normal *A. aegypti* with those having oocysts of *P. gallinaceum* on the midgut at the time of the second blood meal. In both experiments the infected groups of mosquitoes had higher proteinase activity than the uninfected groups. The maximum proteinase activity in all six infected groups occurred 36 hours after the blood meal while in the two uninfected groups it occurred at 24 hrs for one and at 36 hours for the other. Yang and Davies (1971) found that trypsin activity but not chymotrypsin activity in the midguts of *A. aegypti* rose after a blood meal. Combres et al (1971) reported that adult *A. aegypti* have lower chymotrypsin activity than the larvae.

Although the complete mechanism for the control of proteinase secretion in the mosquito is not known, it is clear that serum proteins stimulate proteinase secretion in *A. aegypti* (Shambaugh, 1954). The greatest secretion results when mosquitoes consume a mixture of serum proteins, but the presence of any one serum protein in the meal also stimulates secretion.

Fisk and Shambaugh (1952) and Shambaugh (1954) began studies to elucidate the mechanism which controls the production of midgut proteinase in *A. aegypti* following a blood meal, but attempts to stimulate enzyme secretion by injection of hemolymph from fed to unfed mosquitoes failed. Detinova (1962, p. 59), from studies of digestion and ovarian development in *Anopheles maculipennis* concluded that "the process of ovarian development slows down the speed of digestion. Neurohormonal regulation of the duration of the digestive process may therefore be postulated for mosquitoes." Autogenous *Aedes atropalpus* generally do not take a blood meal during the first gonotrophic cycle (Hudson, 1970). Those which feed utilize neither the protein nor the carbohydrate in the blood meal during production of the first batch of eggs. The results reported by Detinova (1962) and Hudson (1970) suggest that mosquitoes with mature, or nearly mature, eggs are incapable of synthesizing or releasing normal quantities of digestive enzymes.

The amount of midgut proteinase present in *A. aegypti* 27 to 28 hours after engorging on chicks is much lower in mosquitoes decapitated within six hours of feeding than in dewinged individuals (Gooding, 1966b). These results are consistent with but not proof of, humoral control of proteinase secretion. However, using net synthesis of triglycerides as a criterion for digestion of the blood-meal, Lea (1967) concluded that ablation of the median neurosecretory cells of mosquitoes does not affect digestion or absorption. The results of experiments with 5-FU (Akov, 1965) suggest, but do not prove, that the midgut proteinase secreted by *A. aegypti* is formed *de novo* after the ingestion of the blood meal.

In *Simulium venustum* the trypsin activity is higher in midguts of blood-fed females than in females fed on sucrose only (Yang and Davies, 1968b). In *Simulium rugglesi* fed on a duck, the trypsin level rises steadily for 18 hours after feeding, and remains essentially unchanged at 24 hours. In *Prosimulium decemarticulatum* feeding on a chicken, the trypsin level rises sharply by 5 hours then drops slightly during the next 19 hours. *S. venustum* adults feeding on a 50% human blood - 0.5 M sucrose solution, and kept at 15 C nearly double their trypsin activity within 24 hours and maintain this level for about 8 days (Yang and Davies, 1968b). At 30 C the trypsin level is nearly triple that of sugar fed controls and is also maintained at this elevated level for 8 days. Males feeding on the blood-sucrose mixture and maintained at 15 C have a slightly depressed trypsin level for 4 days. *S. venustum* females feeding on duck erythrocytes have only slightly less trypsin than those feeding upon whole duck blood; the reverse is true when feeding on material of bovine origin. The blood meal stimulates trypsin secretion in the midgut of *P. decemarticulatum*, *S. rugglesi* and *S. venustum* (Davies and Yang, 1968).

In *Stomoxys calcitrans* the proteinase activity reaches a maximum about 13 hours after a blood meal but remains essentially unchanged after a sucrose meal (Champlain and Fisk, 1956). No depletion of the midgut proteinase below the level of unfed flies occurs. These authors attributed this difference in pattern between *A. aegypti* and *S. calcitrans* to the different ways in which these species distribute the blood meal between the midgut and crop diverticula.

There is a positive linear relationship between meal size and proteinase activity in the midgut of *Glossina morsitans* at 6, 18, 24, 48, 72, and 96 hours after the blood meal (Langley, 1966a). When the concentration of defibrinated blood in the meal is varied from 10% to 100% but the specific gravity remains relatively constant (1.00-1.05), the amount of proteinase activity varies with the volume of the meal and not the concentration of the blood in the meal. However, since flies consuming saline alone do not secrete proteinase it appears that some blood must be present for proteinase to be produced. The stimulus for proteinase production is in the serum rather than in the erythrocyte. Langley proposed that stimulation of stretch receptors in the crop duct causes impulses to pass along the oesophageal nerves to the neuroendocrine system, resulting in the production and/or release of hormones which cause the middle portion of the midgut to produce the precursors of the proteinase. He further suggested that the enzyme is then activated by some component of the serum portion of the meal. The frequency of feeding of *G. morsitans* (every 48, 72 or 96 hours) does not affect the production of proteinase (Langley, 1969a). Females have a higher maximum proteinase level in the midgut than males. In females the maximum amount of proteinase occurs 24 to 48 hours after a meal while in males it occurs between 12 and 24 hours after the meal. The maximum level of proteinase in field caught males is about 1.5 times the maximum level in laboratory reared males. This latter finding is consistent with the fact that field caught flies excrete the blood meal more rapidly than laboratory reared flies (Langley, 1966b, 1967a).

The amount of proteinase in the midgut of unfed *G. morsitans* rises, during the first 24 hours of adult life, remains constant until the fly is 96 hours old, and declines by 120 hours, the flies die from starvation by 144 hours (Langley, 1967b). The rise in proteinase activity during the first 24 hours is not caused by either crawling up through sand or flight activity. Results of experiments involving puncturing the ptilinum, and injecting material into the teneral fly suggest that distension of the crop (possibly in combination with the presence of protein in the crop) is responsible for the rise in proteinase activity. Experiments involving ligaturing, nerve sectioning, and injection of tissue homogenates, demonstrated that the brain is involved in the production and/or secretion of the midgut proteinase in the unfed teneral fly.

During the first 24 hours of adult life in *Glossina austeni* there is a 50% increase in the midgut proteinase (Langley and Abasa, 1970). This increase occurs in normal males and females and in flies irradiated with 10 krad as pupae. Twenty-four hours after the first blood meal there is a positive correlation between meal size and the amount of midgut proteinase activity. The slope of the regression line is not significantly affected in flies given 10 krad as pupae or 15 krad as 0 to 3 hr old adults. However, the irradiated flies have much lower correlation coefficients (0.49) than the unirradiated controls (0.74), indicating greater variation in the irradiated flies.

Schaefer (1968), largely on the basis of work by Langley (1967a) proposed that the reactions of hosts in the wild, to attack by blood-sucking insects cause stress in the latter. This stress heightens the activity of the neurosecretory processes of the brain and corpora cardiaca and the resulting neurosecretions stimulate the early release of proteolytic enzymes into the midgut.

The invertase activity of *A. aegypti* midguts increases from 2 to 4 hours after the mosquitoes feed on blood (Fisk and Shambaugh, 1954). A depression in invertase activity in both the midgut and diverticula after a sugar meal persists for at least 24 hours. The invertase activity is always higher in the midgut than in the diverticula regardless of the nature of the meal or the time after feeding, indicating that the midgut is the source of this enzyme.

There is no significant difference in the invertase content of sucrose-fed and water-fed *S. venustum* but the invertase level rises immediately after a blood meal and remains elevated for 48 hours (Davies and Yang, 1968; Yang and Davies, 1968c).

Some of the amylase activity in *A. aegypti*, *Culex pipiens*, *S. venustum* and *Simulium vittatum* occurs in the midgut, but most occurs elsewhere principally in the hemolymph (Yang and Davies, 1968a). With *A. aegypti* there is a 3 to 4 fold increase in amylase activity immediately after feeding on man; this activity declines as the blood is digested. By comparing the amylase activity of blood-fed mosquitoes and human blood Yang and Davies concluded that most of the amylase activity in the fed mosquito came from the blood meal.

Freyvogel, Hunter, and Smith (1968) suggested that esterases demonstrated in the midgut of *Anopheles freeborni*, *Anopheles stephensi* and *A. aegypti* may have a role in digestion of the blood meal.

Longevity studies indicate that *A. aegypti* can utilize the disaccharides sucrose, maltose, trehalose, and melibiose, the trisaccharides raffinose, and melizitose, and the polysaccharide dextrin, but not the disaccharides lactose or cellobiose, the polysaccharides starch, glycogen, or inulin or the glycosides α -methylglucoside or α -methylmannoside (Galun and Fraenkel, 1957). Homogenates of whole *A. aegypti* hydrolyze sucrose, maltose, trehalose, raffinose, melizitose, and dextrin but not melibiose, lactose, cellobiose, starch, glycogen, inulin, α -methylglucoside or α -methylmannoside. The results of the feeding experiments and the enzyme tests are consistent except in the case of melibiose, for which no hydrolytic enzyme could be demonstrated.

RELATIONSHIP OF DIGESTIVE PROCESSES TO VECTORING ABILITY.

The discovery of insect transmission of vertebrate pathogens was followed within a few years by the demonstration of numerous examples of vector-parasite specificity. An early hypothesis advanced to explain this phenomenon was that the digestive processes of the insect determine which parasites develop. This hypothesis has been investigated several times beginning with Nuttall (1908) and has remained an attractive explanation. Although tested several times without experimental confirmation, the role of the gut in vector-parasite specificity is still occasionally mentioned. For example Day and Waterhouse (1953a) in a review article stated that "The physiology of the mosquito midgut is of exceptional importance in that it is one of the factors controlling the establishment of malarial parasites within the insect vector". In considering the differences in the digestive rates of *Anopheles sacharovi*, *Anopheles maculipennis*, and *Anopheles superpictus*, Bates (1949, p. 90) wrote "It has been suggested that such specific differences in the digestive process might be a factor in determining the susceptibility of a mosquito to plasmodium invasion." Wigglesworth (1930) wrote "there is, at the present time, a common but indefinite impression that some simple demonstrable difference in the chemistry of the digestive tract (for instance, in salt content or in hydrogen-ion concentration) may be at the back of specificity in the insect host as a vector of pathogenic micro-organisms. Although of course it cannot be denied that this may be so, I do not myself see any *a priori* reason why specificity in the insect host should be due to causes any less subtle than say natural immunity among vertebrates."

The influence of agglutinins can be seen by comparing the distribution of *Plasmodium*

oocysts in the midguts of *A. maculipennis* and *Anopheles stephensi* (Shute, 1948). Both species have anticoagulins but only the former has an agglutinin which causes the red blood cells to clump and settle out. As a result when *A. maculipennis* is in its normal, head-up, vertical position after a blood meal, the erythrocytes settle to the posterior part of the midgut and most *Plasmodium* oocysts are found in this region. However, in *A. stephensi* the erythrocytes do not settle out and the oocysts are more or less uniformly distributed over the midgut.

Lavoipierre (1958) reviewed the relationships between filarial nematodes and their arthropod vectors, including a brief discussion of the possible role of digestive physiology of the vector in limiting the intensity of infection.

Chamberlain and Sudia (1961), in a review of virus transmission by mosquitoes, listed several hypotheses to explain the "gut barrier" to infection including two related to digestive physiology (virus inactivation by digestive fluids and impermeability of the PM). They emphasized that arguments could be presented to support or refute each hypothesis and that no mechanism has been completely proved. For example the suggestion that digestive fluids inactivate the virus can be argued against on the grounds that viable WEE virus is detectable in mosquito midgut for as long as a day after the infectious meal. On the other hand, since digestion commences at the periphery of the blood meal, the virus particles next to the gut wall may be inactivated while those deeper in the clot may be unaffected. Similarly, the pore sizes of the peritrophic membrane may explain differences in susceptibility to viruses of different sizes but not to those of the same size.

Young *Cimex lectularius*, feeding upon mice infected with *Pasturella pestis* (Lehmann and Neumann) fail to reduce the size of the ingested meal within a few days and usually die (Bacot, 1915). Bacot stressed that digestion of blood is very rapid in the midgut of the flea and that with the destruction of both the red blood cells and the leucocytes, the midgut becomes very much like an artificial culture medium in which *P. pestis* may develop rapidly. However, in the crop of the bug *P. pestis* development "differs generally from that which takes place in the stomach of the flea in respect of its slower and looser growth, this limitation of activity being accompanied by and possibly due to the preservation of the structural character of the blood for many days after its ingestion into the crop".

Duncan (1926) ran tests for bactericidal activity in the gut contents and feces of several blood-sucking insects (*Stomoxys calcitrans*, *Anopheles bifurcatus* (L.), *Aedes cinereus*, *C. lectularius*, *Rhodnius prolixus*) and on blood fed *Musca domestica* L. Activity was found against 8 of the 18 species of bacteria used. The bactericidal material from *S. calcitrans* was heat stable (100 C for 30 minutes) and was not destroyed by trypsin. St. John, Simmons, and Reynolds (1930) found no evidence of bactericidal material in the digestive tract of *Aedes aegypti*.

Packchianian (1948a) found that *Leishmania tropica* (Wright) and *Leishmania donovani* fed to several species of *Triatoma* (*T. gerstaeckeri*, *Triatoma lectularia* (Stal) (= *T. heidemannii*), *T. protracta* (Uhler), and *T. uhleri* Neiva) died in the gut within 3 days. Similarly he showed that *Trypanosoma brucei* Plimmer and Bradford, *Trypanosoma gambiense* Dutton, and *Trypanosoma evansi* (Steel) (reported as *T. hippicum*) died within 10 days of ingestion by *Triatoma* spp. (*T. gerstaeckeri*, *T. sanguisuga*, *T. protracta* and *T. rubrofasciata*) (Packchianian, 1948b).

The spirochaete *Treponema pertenuae* Castellani remains mobile much longer in the oesophageal diverticulum than in the stomach of *Hippelates pallipes* indicating that this spirochaete may be affected by the fly's digestive secretions (Kumm, Turner, and Peat, 1935).

A possible influence of defecation, during the act of feeding, upon intensity of infection was shown by Kartman (1953a) who fed five species of mosquitoes (*A. aegypti*, *Aedes*

albopictus, *Culex pipiens quinquefasciatus*, *Culex pipiens* and *Anopheles quadrimaculatus*) on a dog infected with *Dirofilaria immitis* (Leidy). As the mosquitoes became replete, the only species observed to defecate a drop of fluid was *A. quadrimaculatus*. By counting the microfilaria in this drop and in the midguts of the fed mosquitoes, it was estimated that about 7% of the microfilaria were lost from the midgut by defecation.

Kartman (1953b) showed that the clotting of the blood in the mosquito midgut (in for example *Aedes* and *Culex*) reduced the number of microfilaria of *D. immitis* which could leave the midgut. Degeneration of microfilaria in the midgut was observed, and although it was concluded that this destruction was due to the digestive process, it was pointed out that death may not have been caused by the digestive enzymes but rather by other factors in the midgut or salivary secretion.

Huff (1927) fed *C. pipiens* and *Aedes sollicitans* upon canaries infected with *Plasmodium cathemerium* and, at various times after the meal, made smears of the gut contents and observed the appearance of the erythrocytes and the sexual and asexual forms of the parasites. In *C. pipiens*, a susceptible species, the asexual forms began to stain abnormally after 3 hours, and after 6 hours were not found. In *A. sollicitans* the asexual forms had not disappeared after 6 hours and some were found until the end of the series 20 hours after feeding. The fate of the sexual forms was the same as that of the asexual forms. Huff also injected homogenates of the midguts of both species into normal birds at intervals after the mosquitoes had fed on an infected bird. The resulting infections in the birds showed that the asexual forms lose their infectivity after 5 or 6 hours in the gut of both the susceptible and the refractory species. During this study Huff noted that some mosquitoes of both species differed greatly from the others in the rate of digestion of blood. Huff (1934) attempted to determine whether intraspecific variation in the rate of digestion was correlated with the degree of susceptibility to malaria parasites in *C. pipiens*. The mosquitoes were fed upon canaries infected with either *P. cathemerium* or *Plasmodium relictum*. A second meal on an infective bird was given 5 to 8 days later, the mosquitoes then being dissected and their midguts removed and examined microscopically. With this technique, each mosquito served as its own control – those which had large oocysts were susceptible, and the others were considered refractory. No differences were observed in digestion in the susceptible and refractory individuals.

However, de Buck, Schoute, and Swellengrebel (1930, 1932), and deBuck, Torren, and Swellengrebel (1933), suggested that refractoriness in *A. maculipennis* was correlated with slow digestion of the blood meal in certain varieties during overwintering. Ookinetes were formed in both the undigested meal of the “long winged” and in the partially digested meal of the “short winged” mosquitoes and it was proposed that the difference in susceptibility may be due to differences in the ease with which ookinetes can work their way out of the blood meal.

Bishop and McConnachie (1956) found no evidence that exflagellation of *Plasmodium gallinaceum* took place any faster in the stomach of *A. aegypti*, a susceptible mosquito, than under a coverslip on a glass slide.

Attempts have been made to correlate the development of oocysts with the diet of the mosquito. The first was the observation that *Anopheles*, fed alternately upon bananas and gametocyte carriers, often failed to digest their blood meal or to develop oocysts (Darling, 1910). Feeding several salts, including 0.1 M solutions of CaCl_2 or MgCl_2 to *A. aegypti* decreased the number of *P. gallinaceum* oocysts developing on the gut wall (Terzian and Stahler, 1960). However, the same study showed that MgCl_2 fed at a concentration of 0.4 M increased susceptibility to *P. gallinaceum*. The presence of CaCl_2 and MgCl_2 in the diet of *A. aegypti* and *A. quadrimaculatus* inhibited the *in vivo* digestion of blood (Terzian, 1958,

1963), and these same salts had an inhibitory effect upon the *in vitro* activity of *A. aegypti* proteinase (Wagner, Tenorio and Terzian, 1961; Gooding, 1966a). On the other hand, feeding a chloramphenicol-dihydrostreptomycin-sugar solution to *C. pipiens quinquefasciatus* inhibited digestion of blood by this species, but increased its susceptibility to *P. relictum* (Micks and Ferguson, 1961). There was a slight increase in the per cent of the mosquitoes infected (74% in controls, 85%, in treated mosquitoes) and a considerable increase in the intensity of the infection (56 oocysts/midgut in controls and 110 oocysts/midgut in the antibiotic treated mosquitoes).

Attempts have been made to influence the level of infection by decapitating the mosquitoes after a blood meal. Rozeboom (1961) found a decrease in the percentage of mosquitoes infected, in the number of oocysts per midgut, and in the size of the oocysts (*P. gallinaceum*) when *A. aegypti* were decapitated within 6 hours of feeding. These results may indicate a lower nutritive environment for the *P. gallinaceum* oocyst in the decapitated mosquitoes, since decapitation was subsequently shown to influence the amount of proteinase in the midgut of *A. aegypti* (Gooding, 1966b). However, Rozeboom (1961) stated that "blood digestion does proceed in the decapitated females which survive 3 to 5 days. In many specimens a residue of the blood meal may remain in the gut; in others the gut becomes completely empty as in normal mosquitoes. Thus a sufficient degree of normal digestive processes, including changes in the epithelium, continued to take place in decapitated mosquitoes to permit a somewhat reduced number of zygotes to be taken up by the epithelium". Yoeli, Upmanis, and Most (1962) found that decapitation of *A. quadrimaculatus* after a blood meal did not interfere with the normal development of the larvae of *D. immitis*.

Stohler (1961) considered in some detail the relationship between the PM of Diptera and the role of the latter as vectors of blood parasites. He concluded that the PM could influence the intensity of an infection by imprisoning a portion of the parasites within the blood meal but that the parasites can usually escape from the meal through the viscous portion of the PM, through its open, posterior end or at the time the PM breaks up if the parasites have not already been killed. He stated, however, that the full role of the PM in influencing vectoring ability of flies was not completely resolved, and that a study of closely related species which differed widely in their vectoring abilities could be very useful.

Bates (1949, p. 229) considered the critical stage in determining the susceptibility of the mosquito to be the penetration of the gut wall by the ookinete. Mariani (1961) suggested that the PM in *Anopheles labranchiae* Falleroni may hinder the passage of malaria zygotes from the blood meal into the mosquito. He discussed the importance of the PM in vectoring ability of mosquitoes.

Ookinetes of *P. gallinaceum* that fail to penetrate the PM between 20 and 30 hours after *A. aegypti* feeds will perish. After considering the frequency with which ookinetes are found near the PM, Stohler (1957) suggested that the PM constitutes a physical barrier to the penetration of the ookinetes and that penetration of the gut epithelium does not constitute a significant barrier to infection. During the early stages of its formation, the PM does not constitute a barrier to ookinete penetration, but with subsequent hardening it becomes progressively more impenetrable.

Interpretation of the role of digestion in vector-plasmodia specificity is complicated by our uncertainty about the course of development of the malaria parasite within the gut of the mosquito and by the paucity of information on the stage at which development of the parasite stops. Most of the evidence suggests that decreased susceptibility is correlated with a decreased rate of digestion. It thus appears that if the digestive processes of the mosquito play a role in the infection of the mosquito by plasmodia, it would be by providing nutrients to the developing parasite. The possibility that the digestive enzymes of a refractory species

act directly on the parasite has, however, not been adequately investigated and thus cannot be eliminated at this time.

The influence of the PM upon the establishment of *Trypanosoma grayi* (Novy) in *Glossina palpalis* has been studied by Hoare (1931). *T. grayi* are confined to the midgut lumen by the PM for 2 or 3 days after an infective meal. They then migrate back to the hind gut and escape from the PM taking up residence between the PM and the gut wall by 6 to 8 days after the infective meal and eventually migrating forward in this space. The trypanosomes continue to occupy this space for the remainder of their residence in the tsetse fly. Lewis (1950) observed that the blackfly PM prevented many microfilaria from entering the body cavity and he concluded that "Frequently therefore, the membrane protects the fly itself from heavy infection without preventing it from transmitting the parasite".

CONCLUDING REMARKS

The blood-sucking insects are parasites ingesting a meal which is well defined both in respect of its composition and the time it is consumed. As such these insects should be ideal for studies of digestive physiology and nutrition as they relate to parasitism in general and host-parasite relationships in particular.

The size of a blood-sucking insect ultimately determines the size of the blood meal it can ingest. In general the blood meal is rather large compared with the size of the insect, and all the greater when one considers it in relation to the amount of tissue available for synthesis of the digestive enzymes and absorption of the products of digestion. It then appears that blood-sucking insects can digest relatively larger quantities of blood at a time. This however is more apparent than real for most of them have some mechanism which limits the amount of blood being digested at any time. In some there are no anticoagulins and the blood clots in the midgut, while many of those which do have anticoagulins also have agglutinins. The meal is further concentrated by removal of water during or just after feeding. Enzymes are then secreted onto the surface of the meal. In bugs and many of the higher flies most of the meal is stored (and in some species concentrated) in the anterior part of the midgut without any digestion taking place and then passes to the posterior part of the midgut in small quantities for digestion. The net result of both of these methods is that only a small portion of the meal is digested at any one time and that the digestive enzymes are never mixed with the total, freshly ingested, meal.

Digestion of only a small fraction of the meal at a time has several advantages to the insect. One advantage is that if the enzymes were mixed thoroughly with the entire meal there may be such an excess of substrate that substrate inhibition would significantly reduce the rate of digestion. The proteinase inhibitors in serum may also inhibit most of the digestive proteinases thus reducing the rate of digestion or necessitating secretion of increased amounts of enzyme. Thus another advantage of having only a small portion of the meal exposed to the digestive enzymes is that the serum proteinase inhibitors may be titrated out by the digestive enzymes or destroyed by a concerted attack by the midgut proteinases. A third advantage to digesting small quantities of blood close to the midgut epithelium is that the products of digestion are readily available for absorption rather than having to move from the center of the midgut.

Salivary gland anticoagulins and agglutinins are widespread, but not universal, characteristics of blood-sucking insects. Whether these substances indicate a degree of convergence selected for by the nature of the blood meal or the retention of primitive characters is unknown. It would indeed be interesting to examine the saliva of several non-haematophagous insects for anticoagulins and agglutinins. The specific contribution of sali-

vary agglutinins and anticoagulins to the denaturation and digestion of the blood meal has not been investigated. There are however reports that mosquitoes and tsetse flies can digest blood without saliva. Whether the efficiency of the process is unaltered in surgically modified insects is not definitely established. One might expect some effect on digestion in *Glossina austeni* whose salivary ducts have been cut since a plasminogen activator from the salivary glands probably contributes to clot lysis in the midgut.

The role of digestive physiology in host parasite relations has not been systematically examined. The work on digestion by *Pediculus humanus* indicates that this highly host specific ectoparasite encounters difficulties in digesting guinea pig blood. To what extent these difficulties are peculiar to the *P. humanus* - guinea pig system is unknown. Insufficient work has been done on comparison of digestive physiology of insects varying in the degree of host specificity. Vertebrate serum contains some proteins necessary for the secretion or activation of digestive proteinases and others which inhibit proteinases. The relative concentrations or activities of these two kinds of proteins in various vertebrate sera are unknown, as are the responses of various insects to these. It is conceivable that certain highly host specific blood-sucking insects could have very precise requirements with respect to both the proteinase stimulators and the inhibitors. The fate of these stimulators and inhibitors in the digestive tracts of either specific or non-specific blood-sucking insects is unknown but worthy of investigation.

On the basis of work with synthetic substrates as well as specific inhibitors it appears that most of the proteinase activity in the mosquito midgut is due to a trypsin, with much smaller amounts of chymotrypsin also being present. However, immunological studies indicate that digestion products from at least some blood proteins in the midgut of *Culex pipiens quinquefasciatus* are different from those produced by mammalian trypsin. These findings indicate that either the small amount of chymotrypsin may have a marked qualitative influence upon digestion in the mosquito or that the mosquito trypsin has a different bond specificity than mammalian trypsin when whole proteins are used as the substrate.

In this article I have summarized a substantial portion of the literature on digestion in blood-sucking insects and indicated some areas in which further research would be profitable. In subsequent articles in this series I propose to report on digestion in a variety of blood-sucking insects and on contributions to the solution of some of the problems indicated in this article.

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